Conséquences et évolution de l'autofécondation : une approche expérimentale chez des gastéropodes hermaphrodites d'eau douce

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Qu'il vive !

Dans mon pays, les tendres preuves du printemps et les oiseaux mal habillés sont préférés aux buts lointains.

La vérité attend l'aurore à côté d'une bougie. Le verre de fenêtre est négligé. Qu'importe à l'attentif.

Dans mon pays, on ne questionne pas un homme ému.

Il n'y a pas d'ombres malignes sur les barques chavirées.

Bonjour à peine est inconnu dans mon pays.

On n'emprunte que ce qui peut se rendre augmenté

.

Il y a des feuilles, beaucoup de feuilles sur les arbres de mon pays. Les branches sont libres de ne pas avoir de fruits.

On ne croit pas à la bonne foi du vainqueur.

Dans mon pays, on remercie.

René Char Qu'il vive *(1968)*

Introduction

Les forces évolutives agissant sur les systèmes de reproduction : facteurs génétiques

Contrairement aux organismes à sexes séparés (aussi dits dioïques ou gonochoriques), les organismes hermaphrodites produisent des gamètes mâles et femelles soit successivement pendant leur cycle de vie (hermaphrodisme successif), soit simultanément (hermaphrodisme simultané ; Avise 2011). La majorité des plantes à fleurs sont dans ce dernier cas, mais l'hermaphrodisme simultané est également présent dans tous les grands clades d'animaux (excepté les insectes), même s'il n'y est pas majoritaire (Jarne & Auld 2006). Dans certains cas, par exemple chez les gastéropodes ou les trématodes, l'hermaphrodisme ouvre la possibilité de reproduction uniparentale par autofécondation.

L'hermaphrodisme simultané pose des problèmes évolutifs spécifiques, tels que l'évolution de l'investissement relatif dans les fonctions mâle et femelle ou l'alternative entre autofécondation et allofécondation. Concernant ce dernier point, on observe dans la nature des stratégies préférentiellement ou obligatoirement allofécondantes (par ex., la plante *Solanum carolinense*), des stratégies préférentiellement autofécondantes (par ex., l'arabette *Arabidopsis thaliana* ou le nématode *Caenorhabditis elegans*) et des régimes de reproduction mixtes (combinant autofécondation et allofécondation ; par exemple, le thym *Thymus vulgaris*; Goodwillie et al. 2005). De plus les plantes autogames sont souvent morphologiquement différentes de leurs parentes allogames, ce qui a conduit à la caractérisation d'un « syndrome d'autofécondation » (Sicard and Lenhard 2011). Comprendre cette diversité et les déterminants de l'évolution de ces stratégies est un objectif majeur de la biologie évolutive depuis Darwin (1876).

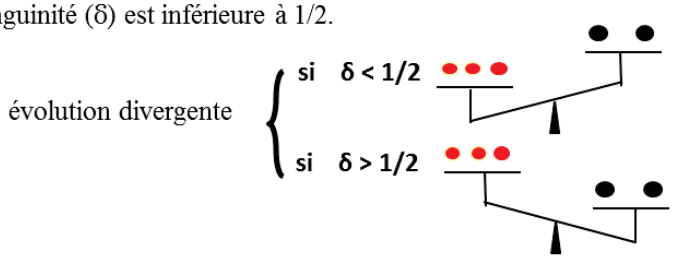
La plupart des modèles génétiques qui ont été développés depuis (revue dans Jarne & Charlesworth 1993 ; Goodwillie et al. 2005 ; Charlesworth & Willis 2009), suggère qu'une

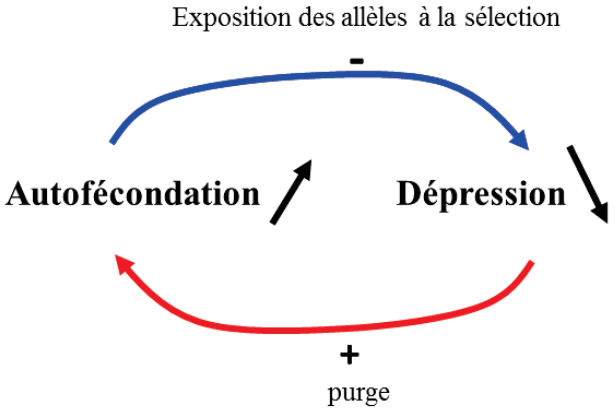
sélection divergente s'opère sur le taux d'autofécondation. En effet, l'autofécondation dispose d'un avantage intrinsèque sous la forme d'une plus forte fidélité de transmission des gènes : un individu autofécondant apparaissant dans une population allofécondante transmet trois copies de gènes quand ses congénères n'en transmettent que deux (Fisher 1941, voir Encadré 1 page 7 sur les avantages de l'autofécondation). A cet avantage s'oppose la dépression de consanguinité (réduction de valeur sélective des descendants d'autofécondation, due généralement à la mise à l'état homozygote d'allèles délétères récessifs ou partiellement récessifs (Charlesworth & Charlesworth 1987, Byers & Wallers 1999, Charlesworth & Willis 2009). Ces deux forces antagonistes conduisent à l'un ou l'autre de deux équilibres stables : l'autofécondation stricte si la dépression de consanguinité est faible, et l'allofécondation stricte si la dépression de consanguinité est suffisante pour annuler l'avantage de transmission. Bien que ce type de modèle considère la dépression de consanguinité comme une constante, celle-ci est susceptible d'évoluer en raison de l'action à long terme de la sélection naturelle sur la fréquence des mutations délétères (voir Encadré 1 page 7 sur les avantages de l'autofécondation). La dépression de consanguinité est alors «purgée» (Byers & Wallers 1999, Charlesworth 2006). En régime d'autofécondation, il est prédit que la sélection purge efficacement les mutations délétères qui sont à la fois d'effet fort et très récessives (Lande & Schemske 1985). Pour des mutations faiblement récessives et/ou faiblement délétères, l'efficacité de la sélection est fortement diminuée, surtout si le nombre de locus impliqués dans la détermination de la dépression est élevé (Charlesworth &Willis 2009, Byers & Wallers 1999, Charlesworth & Charlesworth 1987). En raison de la purge des allèles délétères, et en considérant uniquement les facteurs génétiques, on s'attend à une co-évolution divergente des régimes de reproduction et de la dépression de consanguinité, menant d'un côté à des espèces autofécondantes à faible dépression de consanguinité, et de l'autre à des espèces allofécondantes à forte dépression de consanguinité (Lande & Schemske, 1985).

L'hypothèse semble confirmée par des données empiriques puisqu'on observe aussi bien chez les plantes (Schemske & Lande 1985, Goodwillie et al. 2005, Igic & Kohn 2006) que chez les animaux (Jarne & Auld 2006, Escobar et al. 2011) une tendance vers une distribution en U des taux d'allofécondation (excès d'espèces dont le taux d'autofécondation est proche de 0 ou de 1, voir Encadré 1 sur les avantages de l'autofécondation). Par ailleurs, Husband & Schemske (1996) et Carr & Dudash (1997) observent, en conformité avec les prédictions précédentes, que les espèces/populations autofécondantes ont non seulement une dépression plus faible que les allofécondantes, mais aussi que celle-ci se manifeste pour la survie précoce, stade où s'expriment le plus probablement les allèles létaux et quasi-létaux (mutations perturbant le développement dès son début). Même si moins d'espèces ont été étudiées chez les animaux, on arrive à des observations identiques en tout cas chez les escargots pulmonés (Jarne et al. 2000, Escobar et al. 2011). Les expériences de purge expérimentale (autofécondation ou consanguinité forcée) donnent des résultats assez variables, mais il semble à nouveau que la dépression de consanguinité sur les caractères exprimés précocement dans le cycle de vie diminue en réponse à la consanguinité plus souvent que pour les caractères exprimés tardivement (Byers & Waller 1999, Crnorkrak & Barrett 2002). On observe cependant de nombreuses situations dans lesquelles les taux d'autofécondation sont intermédiaires (Goodwillie et al. 2005). Il parait difficile de considérer qu'il s'agit uniquement de situations de transition entre deux équilibres, comme cela est le cas si l'explication repose uniquement sur des facteurs génétiques (voir Holsinger (1991) pour une exception, puisqu'un régime mixte stable est prédit si l'on prend en compte le « pollen discounting », c'est-à-dire le fait que le pollen utilisé pour l'autofécondation n'est pas disponible pour la fécondation croisée).

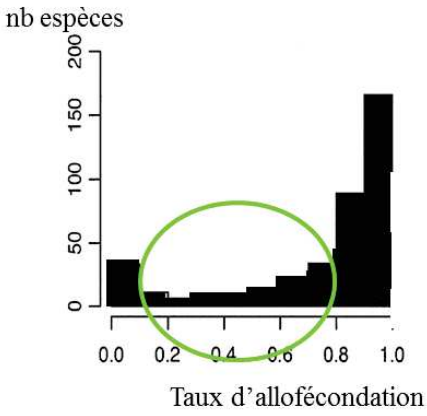
Avantages généralement associés à l'autofécondation

* Un **avantage de transmission** de 3 gènes en autofécondation pour 2 en allofécondation si la dépression de consanguinité (δ) est inférieure à 1/2.





*** Un avantage de reproduction en l'absence de partenaires.



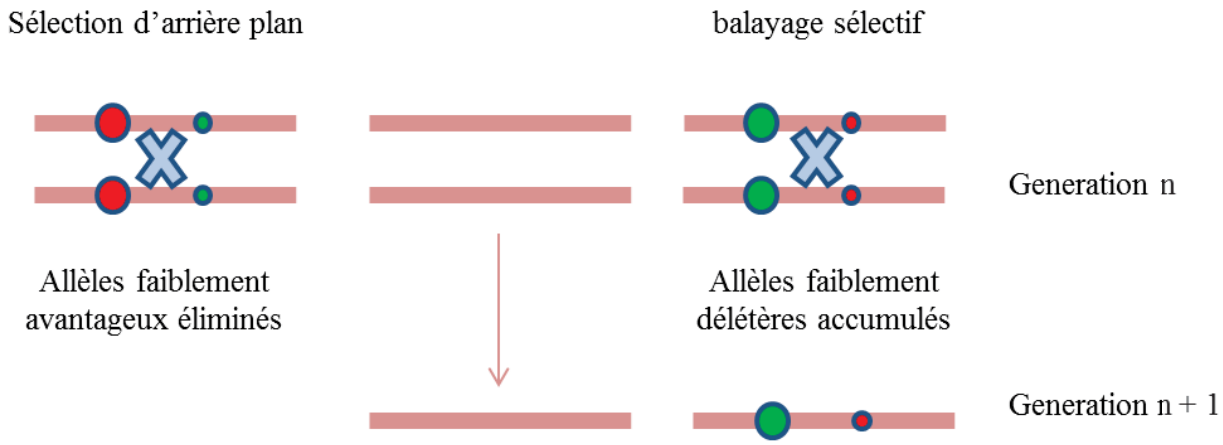
(d'après Igic & Kohn 2006)

Encadré 1. Avantages généralement associés à l'autofécondation.

Désavantages généralement associés à l'autofécondation

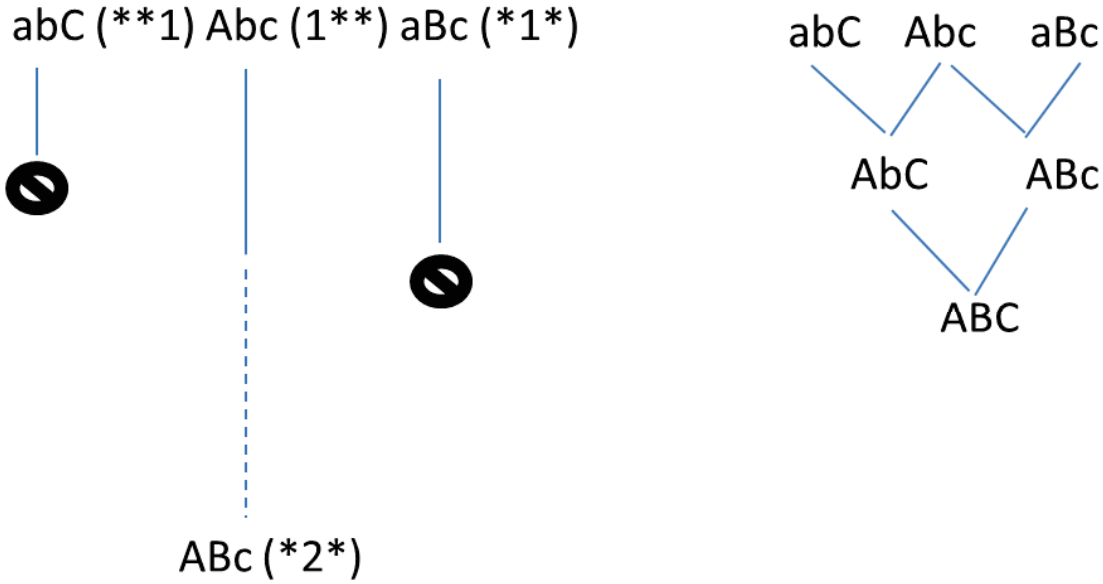
L'autofécondation conduit à une recombinaison inefficace due à la perte d'hétérozygotie, ce qui entraîne au moins deux désavantages majeurs.

1



Les allèles faiblement avantageux liés aux fortement délétères sont éliminés, on a une perte globale de diversité neutre, et une accumulation d'allèles faiblement délétères liés aux allèles avantageux.

2



L'autofécondation comme l'asexualité crée des difficultés pour fixer plusieurs allèles avantageux apparus dans des génomes différents. Il faut souvent attendre la seconde apparition d'un allèle dans un génome qui a déjà fixé la première pour pouvoir avoir les deux, alors que la recombinaison permet la fixation de plusieurs allèles avantageux beaucoup plus rapidement. (D'après Muller 1932)

Encadré 2 : Désavantages généralement associés à l'autofécondation.

Facteurs environnementaux

Plusieurs facteurs environnementaux ont ainsi été évoqués afin d'expliquer la persistance de taux intermédiaires d'autofécondation. Des conditions environnementales générant de faibles densités de population ou un défaut de pollinisateurs favorisent les espèces capables de s'autoféconder, car l'autofécondation procure l'assurance de se reproduire à celui qui la pratique. Parmi ces conditions se trouvent les situations de colonisation (Baker 1955 ; Pannell 2015) : les plantes annuelles, souvent à mode de vie pionnier, sont ainsi plus souvent autofécondantes que les plantes pérennes et présentent une dépression de consanguinité plus faible (Byers & Waller 1999). Des modèles théoriques montrent également que le manque de pollinisateurs à certaines périodes de l'année génère une hétérogénéité temporelle qui peut favoriser des génotypes qui évitent l'extinction dans ces périodes en autofécondant systématiquement une partie de leurs ovules (Porcher & Lande 2005) ce qui aboutit à des taux d'autofécondations intermédiaires (mais élevés) stables.

Transitions évolutives de l'allofécondation vers l'autofécondation : rôle de l'autofécondation facultative

Il est difficile d'observer en temps réel des transitions évolutives d'un régime de reproduction à l'autre, mais on prédit qu'elles devraient se produire plus facilement de l'allofécondation vers l'autofécondation puisque la dépression de consanguinité peut être purgée, ce qui favorise le passage à l'autofécondation. Par contre, aucune force clairement identifiée ne semble pouvoir faire revenir un organisme autogame vers l'allofécondation dès lors que la dépression est purgée. La mutation régénère certes le stock d'allèles délétères, mais il faut maintenir de l'allofécondation majoritaire pendant de nombreuses générations pour que ceux-ci puissent s'accumuler par dérive au point de retrouver de fortes valeurs de dépression.

La faible dépression de consanguinité maintient donc le régime en autofécondation et les deux se renforcent. De plus, chez les plantes au moins, l'évolution vers l'autofécondation implique souvent la rupture de mécanismes complexes favorisant l'allofécondation comme les systèmes d'auto-incompatibilité, qui sont difficile à « ré-inventer » après avoir été perdus, Il en est de même des adaptations morphologiques, comme par exemple la couleur ou la grande taille de fleurs pour attirer les pollinisateurs qui sont coûteuses et inutiles en autofécondation, et qui sont ensuite difficiles à regagner.

Des indices de transitions dans le sens « allofécondation => autofécondation » se retrouvent dans la phylogénie des angiospermes, particulièrement lorsqu'on observe les pertes de fonction aux locus d'auto-incompatibilité, qui semble être suivies rapidement par une augmentation du taux d'autofécondation (Igic et al. 2008) et l'observation d'états transitoires est difficile. Cependant, on observe des formes d'autofécondation facultative qui pourraient constituer un état transitoire entre l'allofécondation stricte et l'autofécondation stricte. Un exemple de ces formes est l'autofécondation retardée (« delayed selfing » ; Lloyd & Schoen 1992), c'est-à-dire intervenant tardivement après un échec de pollinisation chez les plantes ou d'insémination chez les animaux. Chez certaines plantes, la morphologie des fleurs qui fleurissent tardivement facilite l'autofécondation en réduisant la distance entre anthères et stigmates (Kalisz et al. 1999). Chez les gastéropodes pulmonés d'eau douce (Hygrophila) qui se reproduisent préférentiellement par allofécondation, un individu isolé peut retarder sa reproduction, et donc finir par se reproduire par autofécondation, en l'absence de partenaires. La différence entre l'âge auquel il se reproduirait en présence et en absence de partenaires est appelé temps d'attente (« waiting time » ; Tsitrone et al. 2003a, 2003b). Le temps d'attente a été caractérisé chez une quinzaine d'espèces d'Hygrophila, et on observe une relation négative entre temps d'attente et taux d'autofécondation (Escobar et al. 2011). Ce

comportement d'attente a par ailleurs été observé dans d'autres groupes d'animaux hermaphrodites (par ex., des gastéropodes terrestres et des cestodes ; Escobar et al. 2011, Ramm, et al. 2012). Ce « syndrome d'autofécondation » comprend aussi une diminution de l'allocation à la fonction mâle. Chez les plantes, elle peut être inférée à l'aide du ratio pollen/ovule (Cruden 1977, Charnov 1982). Chez les animaux, elle peut l'être via l'investissement dans l'activité copulatoire (Pélissié et al. 2012).

A partir de là, il est possible de proposer un scénario évolutif faisant intervenir l'autofécondation facultative comme intermédiaire évolutif entre allogamie et autogamie (Figure 1). Si une espèce auto-compatible, mais préférentiellement allogame, est confrontée à des périodes défavorables qui conduisent à une réduction de la possibilité d'allofécondation (par ex., absence de pollinisateurs ou de partenaires), cela peut conduire à une augmentation forcée du taux d'autofécondation. Si ces périodes défavorables sont suffisamment longues ou fréquentes, cette autofécondation entraîne une purge de la dépression de consanguinité. Lorsque cette dernière diminue en-dessous du seuil δ (voir Encadré 1 p7), la sélection favorise une autofécondation préférentielle. Cette étape s'accompagnerait (i) de l'amoindrissement des caractères qualitatifs ou quantitatifs déterminant la "résistance" à l'autofécondation (par exemple, auto-incompatibilité ou temps d'attente) et (ii) d'une diminution de l'allocation à la fonction mâle qui perd son utilité suite à l'augmentation de l'autofécondation (par ex., diminution du ratio pollen / ovules chez les plantes ou réduction de l'investissement dans l'activité de copulation chez les animaux).

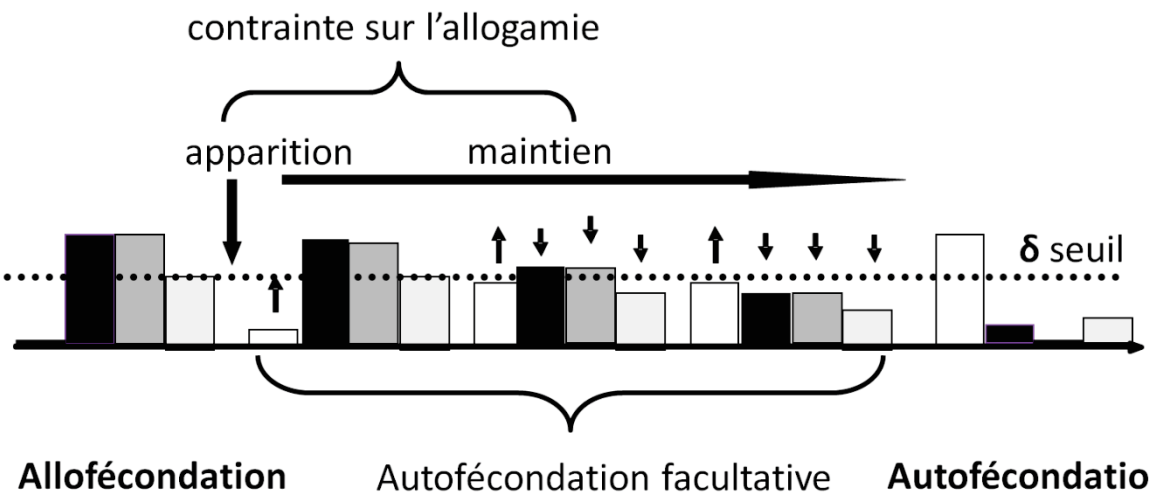


Figure 1. Scénario de transition de l'allofécondation vers l'autofécondation. Sont représentés le taux d'autofécondation (blanc), la dépression de consanguinité (noir), la résistance à l'autofécondation tel qu'un temps d'attente (gris sombre) et l'allocation à la fonction mâle (gris pâle). Le seuil (δ) correspond à la valeur de la dépression en dessous de laquelle l'autofécondation est favorisée.

Attendus lors d'une transition vers l'autofécondation… et après la transition

Si on force expérimentalement l'autofécondation, selon les prédictions théoriques, une diminution du temps d'attente à la reproduction, des indices de purge de la dépression de consanguinité et une diminution de l'allocation à la fonction mâle relativement à la fonction femelle se traduisant par une diminution du succès reproducteur mâle et/ou une augmentation du succès reproducteur femelle. Nous avons testé ces prédictions et les résultats sont décrits dans le Chapitre I.

Une espèce autofécondante est censée avoir un plus faible potentiel adaptatif qu'une espèce allofécondante car l'autofécondation a dans un premier temps réduit le polymorphisme en purgeant la dépression de consanguinité et dans un second temps continue de le réduire car

elle est plus sensible aux interférences sélectives (voir Encadré 2 p8). De plus, elle divise par deux l'effectif et la recombinaison efficace de la population, en supprimant progressivement l'hétérozygotie. Les génomes sont alors soumis à une sélection d'arrière-plan : un allèle avantageux fortement sélectionné peut se retrouver dans le même génotype que des allèles faiblement délétères, qui peuvent se fixer plus facilement en autofécondation (Glémin 2007, Kamran-Disfani & Agrawal 2014). Ainsi, bien que la sélection sur un seul locus soit plus efficace en autofécondation, pour peu que l'allèle avantageux soit récessif (Charlesworth 1992, Caballero & Hill, 1992, Glémin & Ronfort, 2013), elle l'est beaucoup moins qu'en allofécondation si on considère la sélection à plusieurs locus.

On a en effet de plus en plus de données suggérant que le système de reproduction impacte les génomes et leur diversité neutre (Wright et al.2008 pour une revue chez les plantes, Jarne 1995 et Burgarella et al. 2015 pour les animaux, Glémin & Galtier 2012 pour une revue générale des différences entre régimes de reproduction), voire même la longueur du génome ce qui suggère que l'autofécondation favorise la fixation de mutations chromosomiques.

De plus, la sensibilité plus grande aux interférences sélectives (sélection d'arrière-plan et balayage sélectif) et la baisse de l'effectif efficace en autofécondation génèrent une variance génétique moindre. Ce déficit de «standing variation» peut conduire une population à s'éteindre en cas de changement brusque d'environnement (Stebbins 1957, Orr & Unckless 2008), d'autant plus que ce déficit est assorti généralement d'une accumulation de mutations délétères.

On prédit donc que l'autofécondation, est un « cul de sac évolutif. » Cela semble confirmé par l'observation des phylogénies qui ne montrent aucun clade autofécondant dont l'autofécondation est l'état ancestral. De plus, les transitions ne semblent aller que dans un sens : de l'allofécondation vers l'autofécondation (Barrett 2013, Wright 2013, Igic et al. 2006) et les espèces autofécondantes ont un taux d'extinction plus important (Goldberg et al. 2010). Ces prédictions sont difficiles à étayer expérimentalement en raison de l'échelle de temps, mais même sur un temps de quelques générations qui permettrait d'évaluer les étapes initiales, les expériences sont rares (voir néanmoins Morran et al. 2009 sur les nématodes).

Au cours de cette thèse, j'ai testé à l'aide de deux expériences principales sur des lignées d'évolution expérimentale de *Physa acuta*, les deux scénari proposé dans cette introduction : la première, basée sur le scénario décrit dans la Figure 1, concerne les attendus au cours d'une transition de l'allofécondation vers l'autofécondation. L'expérience, ainsi que les résultats et conclusions, est décrite dans le chapitre II. Le second scénario concerne les attendus après une transition en particulier les éventuelles différences de potentiel adaptatif entre allofécondation et autofécondation. Il est testé grâce à une expérience de sélection artificielle sur des lignées d'évolution expérimentale décrite dans le chapitre III. Enfin, le premier chapitre de cette thèse décrit le modèle biologique (Chapitre IA) ainsi que les lignées d'évolution expérimentale utilisées pour ce travail (Chapitre IB).

Chapitre I. Une approche expérimentale

Pour tester la validité du scénario décrit dans la Figure 1, nous avons analysé l'évolution de trois traits associés aux régimes de reproduction chez un escargot

hermaphrodite d'eau douce *Physa acuta*, à savoir la dépression de consanguinité, le temps d'attente (forme de « delayed selfing » chez les animaux), et l'allocation des ressources mâle et femelle quand on contraint la disponibilité en partenaires (Chapitre II). Une approche comparative *in natura* (comparaison entre milieux à forte et faible contrainte) parait *a priori* complexe car (i) il est difficile d'évaluer le degré de contrainte réel sur la rencontre de partenaires - la densité individuelle est en elle-même peu aisée à évaluer, car variant rapidement dans le temps et l'espace (Henry 2003), et sa relation au taux d'autofécondation mal connue ; (ii) il est difficile de séparer la part de variation phénotypique due à la génétique de celles dues à d'autres sources (*i.e.* variation environnementale *sensu lato*) . Nous avons donc opté pour une approche d'évolution expérimentale qui permet de s'affranchir de ces problèmes. Nous sommes bien conscients que l'évolution expérimentale engendre aussi des inconvénients comme entre autres la possibilité de dérive génétique due à l'isolement des lignées et une éventuelle adaptation aux conditions de laboratoire (Garland et Rose, 2009), mais nous avons essayé de les quantifier et de les distinguer des effets attendus du protocole de sélection (voir Chapitre IB).

Dans un second temps, nous avons réalisé une expérience de sélection artificielle sur un trait quantitatif, en manipulant le régime de reproduction, afin de déterminer si la réponse à la sélection peut différer lorsqu'une même espèce est soumise à deux régimes de reproduction (voir Chapitre III), ce qui s'approche de l'idée de quantifier le potentiel adaptatif d'une population en fonction de son régime de reproduction. Nous allons dans un premier temps décrire le modèle biologique et le protocole d'évolution expérimentale, avant de présenter les résultats de la première (Chapitre II) et de la seconde (Chapitre III) expérience.

A - Le modèle biologique, Physa acuta

1. Classification, distribution et habitat

Physa acuta (Draparnaud) (Figure 3) est un gastéropode pulmoné appartenant au sous-ordre des Hygrophila (anciennement Basommatophores), et plus particulièrement à la famille des Physidés. La phylogénie de cette famille qui contient une vingtaine d'espèces a été clarifiée par Wethington & Lydeard (2007). Un important travail mené par R.T. Dillon et collaborateurs a par ailleurs permis de rassembler sous le seul vocable « acuta » un nombre important de noms d'espèces (Dillon et al. 2002, 2005). *Physa acuta* est originaire de la partie ouest des USA, et a colonisé l'ensemble de la planète, des tropiques aux régions sub-boréales, depuis le 18ème siècle, pour devenir de fait l'espèce de gastéropodes d'eau douce la plus cosmopolite (Dillon et al. 2002, Bousset et al. 2014).

Figure 3. Individu de *Physa acuta* adulte dans son milieu. La plus grande longueur de la coquille est de 1 cm environ.

Physa acuta occupe toute la gamme des habitats d'eau douce (par ex., mares, rivières, milieux artificiels …), milieux qui sont souvent fragmentés et qui présentent des degrés d'isolement et d'instabilité très variables. Il s'agit d'un aspect central pour l'hypothèse d'assurance de reproduction – en effet, les tailles et densité de populations peuvent varier de

façon marquée (Henry et al. 2005), incluant des densités de l'ordre du millier d'individus / m^2 comme cela est illustré dans la Figure 4. Les extinctions sont aussi fréquentes, parfois suivies de colonisations en densité initiale faible (Henry et al. 2005 ; obs. pers. en Guadeloupe), ce qui peut nécessiter une reproduction par autofécondation. *P. acuta* supporte bien une large gamme de températures, ce qui est probablement à l'origine de son succès.

Figure 4. Concentration d'individus de *P. acuta* sur une noix de coco dans la mare Gorot en Guadeloupe.

2. Coquille, traits d'histoire de vie et reproduction

La coquille de *P. acuta*, comme celle de tous les Physidae, présente un enroulement sénestre (Figure 3). La taille de la coquille à l'éclosion est d'environ 1 mm ; elle atteint environ 6 à 8 mm de longueur à la maturité sexuelle (i.e., vers 40 jours à 25°C en laboratoire; Jarne et al. 2000, Tsitrone et al. 2003b), et jusqu'à près de 15 mm à la mort (Henry 2002). La largeur est environ la moitié de la longueur. La coquille peut être mesurée relativement aisément à l'aide d'un micromètre inséré dans une loupe binoculaire. La répétabilité de cette mesure a été estimée au-delà de 98% sur la longueur et de 95% sur la largeur.

La morphologie de la coquille est éminemment plastique chez les gastéropodes (voir par ex. Dillon 2000), et la forme, l'épaisseur ou la croissance peuvent répondre à différents stimulus tel que la prédation (Auld & Relyea 2010) ou la présence de partenaires (Tsitrone et al. 2003b). Elle présente cependant une base génétique dont nous avons tiré parti dans cette thèse. Nous avons en effet pu montrer lors d'une expérience préliminaire que l'héritabilité du rapport largeur / longueur (l/L) était de 0,17 estimée sur 400 individus issus de 40 familles de pleins frères. Le protocole permettait de prendre en compte les effets maternels, et ceux-ci n'étaient pas significatifs pour ce trait. Compte-tenu d'une répétabilité élevée et d'une héritabilité « honorable », le ratio l/L est apparu comme un caractère de pertinent pour mener une expérience de sélection sur plusieurs générations.

D'autres aspects importants dans le cadre de ce travail sont les traits de vie et la reproduction. Les individus pondent des masses gélatineuses (pontes) entourées d'une membrane translucide, permettant l'adhésion à un support solide (par ex., morceau de bois en milieu naturel, parois des boîtes en laboratoire) et contenant en moyenne 10 à 20 œufs (Jarne et al. 2000, Escobar et al. 2007, 2009). Les œufs mesurent environ 0,5 mm et peuvent être comptés à l'aide d'une loupe binoculaire. L'incubation dure de 6 à 8 jours en conditions de laboratoire. Le taux d'éclosion dépend du système de reproduction, et peut atteindre 90-95% en fécondation croisée.

La vie sexuelle débute par une phase de protandrie durant quelques jours (Wethington & Dillon 1993), suite à laquelle les individus deviennent sexuellement mûrs pour les deux fonctions. Le mode de reproduction chez *P. acuta* est la reproduction croisée ou allofécondation, même si l'autofécondation est tout à fait possible (Henry et al. 2005, David et

al. 2007, Bousset et al. 2014) – en cela, *P. acuta* est similaire à de nombreux autres gastéropodes d'eau douce (Escobar et al. 2011). L'autofécondation est utilisée dans les situations où les partenaires sexuels sont rares. Dans ce cas, un individu s'autoféconderait après avoir attendu un éventuel partenaire sexuel – le temps correspondant à cette attente est appelé temps d'attente (« waiting time » ; Tsitrone et al. 2003a, 2003b), comme exposé en Introduction, et l'autofécondation peut être considérée comme « retardée » (« delayed selfing »). Le temps d'attente est héritable, associé à une réallocation des ressources vers la croissance et la reproduction future, et dépend de la dépression de consanguinité (Tsitrone et al. 2003b). A cette stratégie d'allofécondation préférentielle est associée une dépression de consanguinité forte, comme l'ont montré diverses études depuis une vingtaine d'années (Jarne et al. 2000, Tsitrone et al. 2003b, Escobar et al. 2007, 2009, 2011). On notera une composante marquée sur la fonction mâle (Jänicke et al. 2013), celle-ci étant d'autant plus forte que la compétition mâle est importante.

Les individus sont matures et sexuellement actif durant à peu près toute leur existence, même si une sénescence de reproduction a été mise en évidence (Auld et al. 2014). Ils peuvent survivre plus de six mois en conditions de laboratoire (Auld et al. 2014), mais aussi en populations naturelles (Henry 2002).

3. Comportements copulatoires

Ces comportements ont été largement décrits chez *P. acuta* par Wethington & Dillon (1996), Facon et al. (2006) et Pélissié et al. (2012, 2014), et sont assez stéréotypés chez les Hygrophila (Jarne et al. 2010). Deux individus vierges, ou n'ayant pas copulé depuis longtemps, mis en présence copulent à la fois en tant que mâle et en tant que femelle au bout de quelques minutes à dizaines de minutes. La copulation est unilatérale, *i.e.* qu'un individu

ne joue qu'un seul rôle sexuel à la fois avec un partenaire donné (l'un jouant mâle, l'autre femelle), ce qui permet d'identifier formellement les rôles sexuels de deux partenaires (Fig. 5).

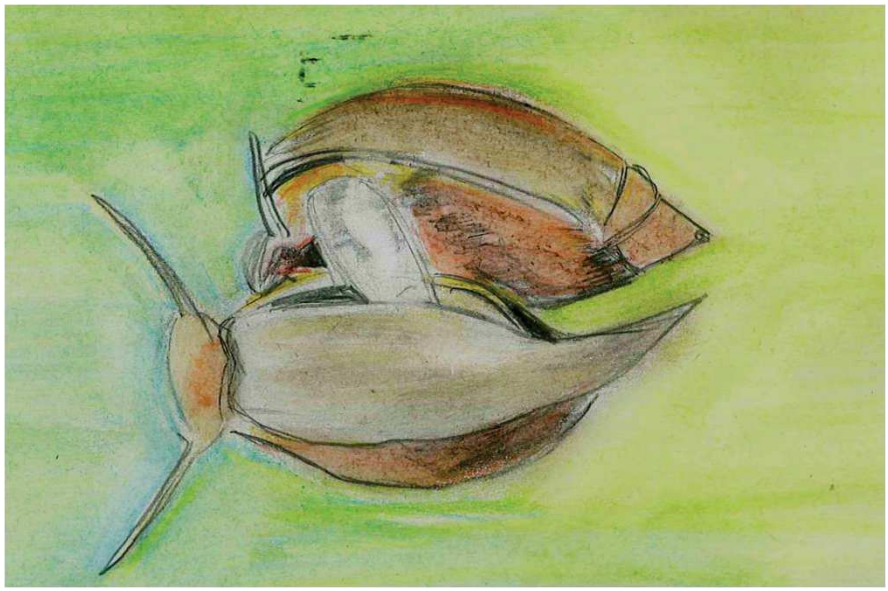


Figure 5. Deux individus en train de copuler l'un jouant le mâle (en dessus) introduisant son pénis dans le gonopore du second (gonopore non visible) par l'ouverture gauche de la coquille.

L'inversion des rôles est possible après une première copulation. L'individu jouant le rôle de mâle transmet du sperme qu'on désigne comme allosperme – le sperme utilisé en autofécondation est appelé autosperme. La plupart des copulations durent entre quelques dizaines de secondes et quelques minutes, et se déroulent selon une séquence comportementale caractéristique (Jarne et al. 2010 ; Pélissié et al. 2012, 2014) – chaque phase peut être quantifiée, par exemple par sa durée. Deux partenaires potentiels entrent en contact. L'un va jouer le rôle mâle en se hissant sur la coquille de son partenaire dans un mouvement circulaire. Un comportement de rejet par l'individu jouant femelle peut avoir lieu. L'individu jouant le rôle mâle dévagine alors son prépuce afin de l'insérer dans le gonopore de son partenaire. Le transfert de sperme n'est pas détectable par simple observation (comme d'ailleurs chez la plupart des espèces pratiquant la copulation). De l'allosperme fécondant

peut être stocké par le récepteur (femelle) pendant plusieurs semaines (Jarne et al. 1993, Dillon et al. 2005). Des copulations multiples peuvent conduire à des paternités multiples (Pélissié et al. 2012, 2014 en laboratoire ; Henry et al. 2005 en conditions naturelles).

4. Méthodes d'élevage

Les individus de *P. acuta* ont été manipulés selon un protocole commun, mis au point lors de travaux menés au cours des 20 dernières années (Jarne et al. 2000, Escobar et al. 2007, 2008, Pélissié et al. 2012, 2014), adapté aux besoins des expériences conduites. La figure 6 fournit un exemple de conditions de manipulation.

Figure 6. Élevage de *Physa acuta* en laboratoire. Les individus sont généralement maintenus dans des boites individuelles de 75 ml, placées sur des plateaux pouvant héberger 30 à 40 boîtes.

Les points importants de ce protocole sont, au-delà des mesures propres à chaque expérience, des conditions expérimentales stables (photopériode artificielle de 12 h jour / 12 h nuit et température de 25°C). L'identification des individus lorsqu'elle est nécessaire, est

basée sur un marquage individuel à l'aide de peinture qui n'affecte pas les traits d'histoire de vie des individus (Henry & Jarne 2007). Les individus sont nourris avec de la salade préalablement bouillie, broyée (plus ou moins finement selon l'âge des individus) et conservée au congélateur. L'eau provient d'un pompage situé sur le campus du CNRS à Montpellier.

B- Evolution expérimentale, revue rapide des points clés de notre protocole

1. Historique des populations et des expériences

Quatre lignées expérimentales de *Physa acuta* élevées en laboratoire depuis 2008 et issues d'un échantillon de populations naturelles d'origine montpelliéraine ont été utilisées : les lignées S (S1, S2) exposées une génération sur deux à l'autofécondation (une autofécondation permanente conduirait à un effondrement des effectifs), et les lignées C (C1, C2) allofécondées à chaque génération (le protocole complet est relaté dans le Chapitre II). Des individus ont été extraits de ces lignées au bout d'une vingtaine de générations environ (G17, G18, G20 et G21 respectivement pour S1, S2, C1 et C2) pour la première expérience (Chapitre II) et d'une trentaine de générations (A1 : G29, A2 : G31, T1 : G35 et T2 : G34) à la fois pour la seconde expérience (Chapitre III), pour l'analyse de marqueurs neutres et pour une mesure de dépression de consanguinité complétant celle faite lors de la première expérience.

2. Polymorphisme génétique neutre et dépression de consanguinité

En parallèle aux études de traits d'histoire de vie, il nous a paru important de caractériser le polymorphisme génétique neutre des lignées que nous avons utilisées dans cette thèse. Pour ce faire, nous avons tiré parti de locus microsatellites caractérisés

antérieurement (Sourrouille et al. 2003, Escobar et al. 2008). Nous avons utilisé sept loci polymorphes et présentant des patrons réplicables (AF108762, AF10874, Pac1, Pac2, Pasu1-2, Pasu1-9, Pasu1-11) selon un protocole utilisé plusieurs fois (Sourrouille et al. 2003, Escobar et al. 2008, Bousset et al. 2014). Les travaux de génotypages ont été menés par A. McKenzie (stage de M1 que j'ai co-encadré) et par A. Segard. La diversité génétique ("gene diversity") a été estimée dans les lignées C et S (Figure 7) à l'aide de Genepop 4.2.2 (Raymond and Rousset 1995) pour les générations indiquées ci-dessus.

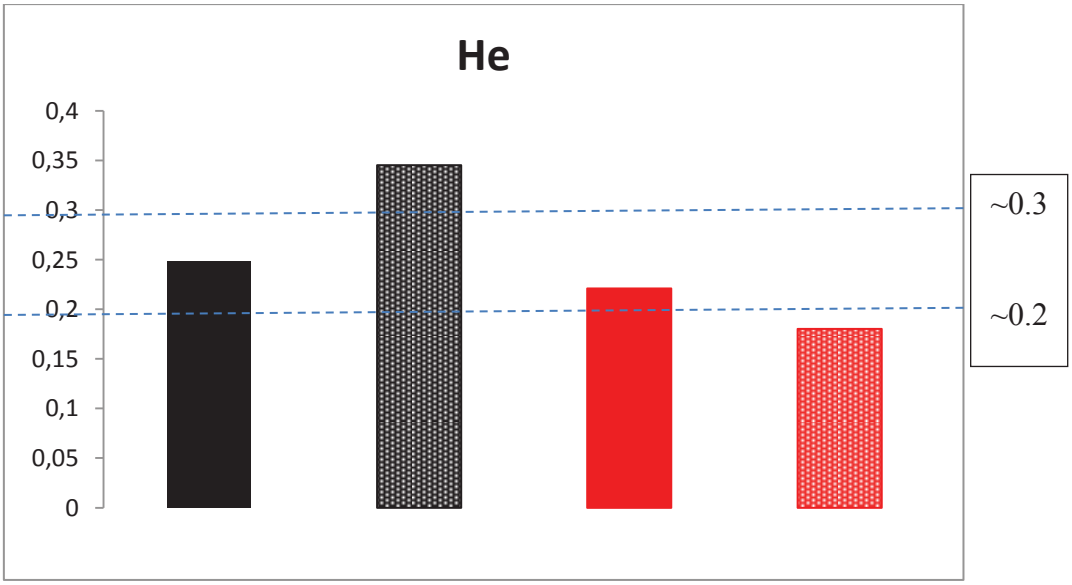


Figure 7. Diversité génétique (He) estimée à l'aide de sept locus microsatellites pour chaque réplicat et chaque type de lignées (en noir plein C1, pointillés C2, en rouge plein S1, pointillés S2). Les lignes bleues en pointillés représentent la moyenne des deux réplicats pour les lignées C et S (respectivement 0,3 et 0,2).

A l'aide de ces mesures, ainsi que de l'estimation du F_{st} moyen entre les réplicats (résultats non montré ici), il est possible d'estimer la diversité neutre moyenne de la population ancestrale commune aux quatre réplicats à l'aide des équations ci-dessous (formules dans Rousset 2013). La valeur inférée est de 0.489 pour cette population.

$$\boldsymbol{Fst(t) = \frac{Q_{intra}(t) - Q_{inter}(t)}{1 - Q_{inter}\,(t)}}$$

$$Fst(t) = 0.513$$

$$He(t) = 0.249$$

$$\boldsymbol{Q_{intra}(t) = 1 - He(t) = 0.751}$$

$$Q_{inter\,(t)} = Q_{intra\,(0)} = 0.489$$

En utilisant les effectifs efficaces (estimés à partir des effectifs nominaux) à chaque génération d'évolution expérimentale, il est possible d'estimer la perte de diversité neutre attendue à la génération 0 de l'expérience de sélection artificielle du Chapitre III, en considérant la probabilité de non coalescence (c'est-à-dire la probabilité que deux gènes pris au hasard dans une population ne soient pas identiques par descendance) comme un indicateur de la diversité neutre. On peut voir sur la Figure 8 que les lignées C et S auraient dû perdre environ ¼ et 1/3 de leur diversité. Si on utilise la valeur estimée au paragraphe précédent, on devrait avoir un He moyen d'environ 0,368 pour les C et de 0,327 pour les S à la génération 0. Or les valeurs estimées sont de 0,3 et 0,2 respectivement (Figure 7), et les pertes de diversité neutre sont plus importantes que prévu. La perte plus importante de diversité des C peut être attribuée à la variance de succès reproducteur entre les individus, et celle des S à la purge de la dépression de consanguinité qui aurait éliminé des familles entières. Ces suggestions sont des hypothèses et il faudrait intégrer au calcul une variance de succès reproducteur et de dépression et voir si c'est compatible avec les valeurs de dépression connue et de succès reproducteur en allofécondation connu dans les populations naturelles. L'idée était de distinguer la perte de diversité attendue uniquement par dérive dans les lignées, de la perte attendue par la pression de sélection sur l'autofécondation imposée pendant le processus

d'évolution expérimentale. Ces résultats sur la variabilité neutre sont importants, mais probablement pas encore complètement exploités dans le cadre des deux expériences menées dans cette thèse - il paraissait donc important de les mentionner. Nous avons conscience que des lignées évoluant expérimentalement en total isolement l'une par rapport à l'autre peuvent avoir des trajectoires totalement différentes même si elles subissent le même protocole de sélection (Teotonio & Rose 2000). Dans notre cas, nous ne nous attendons pas à voir apparaître de nombreuses mutations, et pensons donc que les trajectoires divergent principalement à cause de la dérive.

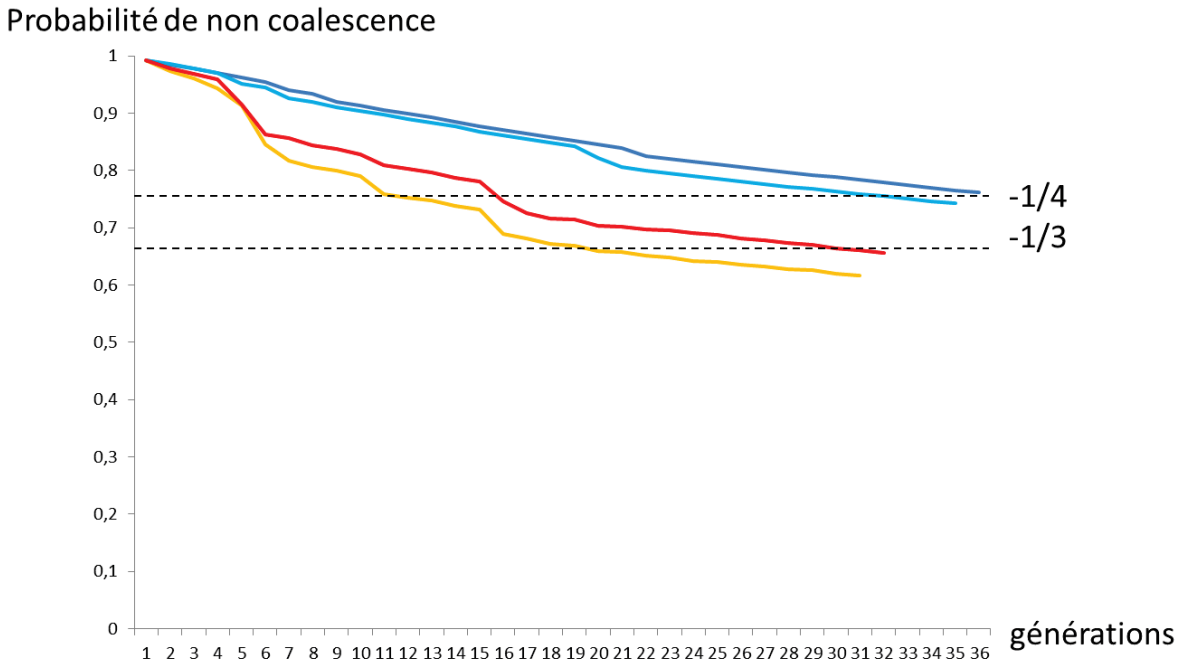


Figure 8. Attendus pour l'évolution de la probabilité de non coalescence sous l'hypothèse neutre (prise en compte uniquement des effectifs à chaque génération) dans les lignées d'évolution expérimentale. Lignées : C1 (bleu foncé), C2 (bleu clair), S1 (jaune) et S2 (rouge).

Un point important est que les lignées S et C étaient encore suffisamment polymorphes au début de l'expérience de sélection artificielle (Chapitre III) pour espérer

disposer de suffisamment de polymorphisme sur le trait phénotypique considéré (forme de la coquille), même si la relation entre polymorphismes neutre et sélectionné n'est pas toujours claire (Frankham, 2012). De plus, nous avons toutes les raisons de considérer que le trait choisi n'était pas sous sélection dans les conditions de laboratoire dans lesquelles nos lignées ont évolué.

Chapitre II Conséquences évolutives de l'autofécondation

Notre première expérience a consisté à mesurer la dépression de consanguinité sur des composantes précoces et tardives, le temps d'attente avant autofécondation et l'allocation aux fonctions mâle et fonction femelle, puis à comparer ces traits entre type de lignées (C et S). Les résultats de cette expérience sont entièrement relatés dans le Chapitre II. Nous avons trouvé une purge significative de la dépression de consanguinité sur la survie juvénile des descendants (figure 9). Celle-ci a été confirmée lors d'une nouvelle mesure en 2014 lors d'un stage de Master ; un résultat curieux est un fort effet réplicat, le réplicat S1 indiquant une dépression fortement négative (Figure 9) pour laquelle nous n'avons pas d'explication convaincante mais qui offre des perspectives intéressantes du point de vue de l'architecture de la dépression de consanguinité.

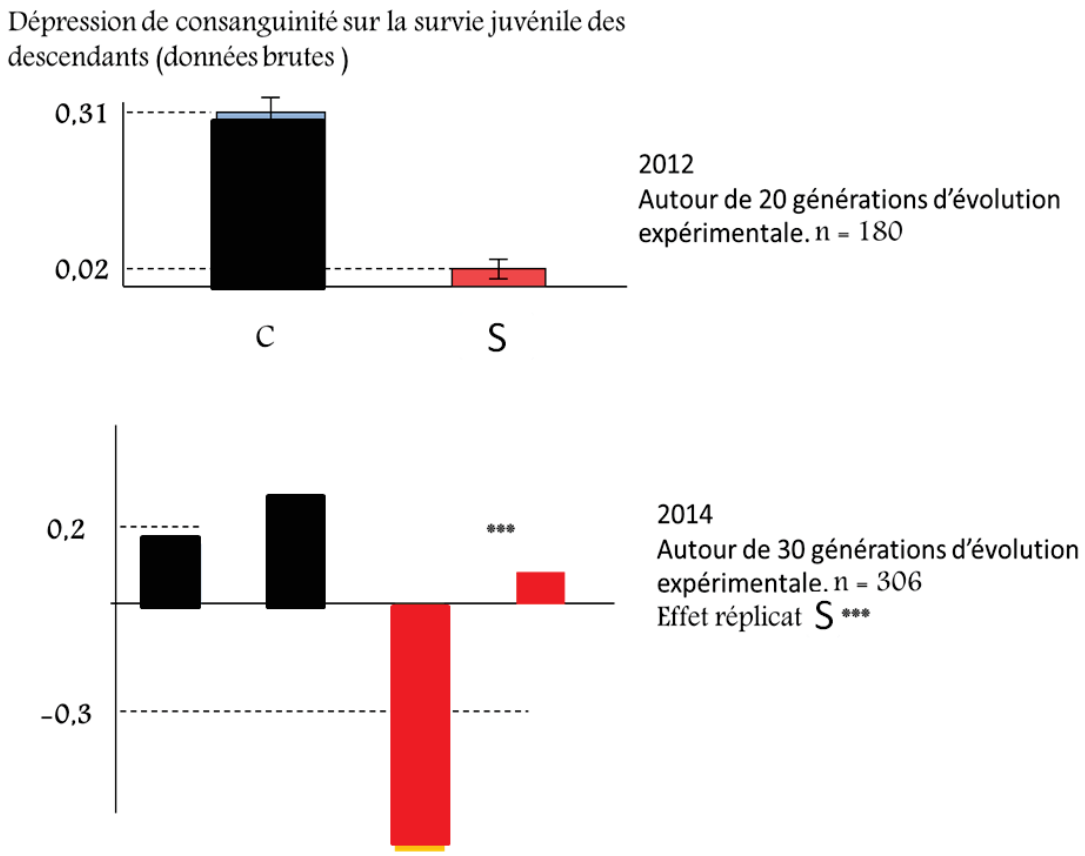


Figure 9. Dépression de consanguinité moyenne mesurée (1-(survie en allofécondation/survie en autofécondation)) dans les lignées C (en noir) et les lignées S (en rouge) lors de deux expériences (2012 et 2014 ; le résultat de 2012 est aussi reporté dans la suite du chapitre II). Les valeurs des réplicats n'étant pas significativement différentes, le graphique a été réalisé avec les moyennes pour chaque lignée, les lignes pointillées indiquent ces moyennes sur l'axe des ordonnées. En bas, les résultats sont reportés pour chaque réplicat, les traits pointillés indiquant la moyenne des deux réplicats pour chaque lignée. La dépression est négative pour le réplicat S1 en 2014, ce qui indique que la valeur sélective des descendants d'allofécondation est inférieure à celle des descendants d'autofécondation (*i.e.* dépression d'allofécondation).

Reduced mate availability leads to evolution of self-fertilization and purging of inbreeding depression in a hermaphrodite

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Author contributions: EN, PJ & PD designed the study. BP initiated the experimental evolution protocol and PD & VS followed the experimental lines until the beginning of the experiment. EN, PD, YC and TJ performed the experiments. EN, PJ & PD analyzed the results and wrote the paper.

Abstract

Basic models of mating-system evolution predict that hermaphroditic organisms should mostly either cross-fertilize, or self-fertilize, due to self-reinforcing coevolution of inbreeding depression and outcrossing rates. However transitions between mating systems occur. A plausible scenario for such transitions assumes that a decrease in pollinator or mate availability temporarily constrains outcrossing populations to self-fertilize as a reproductive assurance strategy. This should trigger a purge of inbreeding depression which in turn encourages individuals to self-fertilize more often and finally to reduce male allocation. We tested the predictions of this scenario using the freshwater snail *Physa acuta*, a self-compatible hermaphrodite that preferentially outcrosses and exhibits high inbreeding depression in natural populations. From an outbred population, we built two types of experimental evolution lines, controls (outcrossing every generation) and constrained lines (in which mates were often unavailable, forcing individuals to self-fertilize). After ca. twenty generations, individuals from constrained lines initiated self-fertilization earlier in life and had purged most of their inbreeding depression compared to controls. However, their male allocation remained unchanged. Our study suggests that the mating system can rapidly evolve as a response to reduced mating opportunities, supporting the reproductive assurance scenario of transitions from outcrossing to selfing.

Introduction

Basic genetic models of mating-system evolution predict that selfing rates in hermaphrodites should evolve either towards either 0, or 100%, depending on whether inbreeding depression (ID) compensates, or not, the intrinsic transmission advantage of self-fertilization (Fisher, 1941). Although early models assumed that ID itself did not evolve, later work demonstrated that relaxing this assumption only reinforced the conclusion (review in Goodwillie et al. 2005; Charlesworth and Willis 2009). Specifically, self-fertilization exposes deleterious alleles to selection promoting ID purging. Thus the selfing rate and ID are expected to be poised in a coevolutionary loop ending in either of two stable states: high outcrossing with high ID, or high selfing with low ID (Lande and Schemske 1985). In agreement with this idea, empirical distributions of outcrossing rates in both plants (Schemske and Lande, 1985; Goodwillie et al. 2005; Igic and Kohn, 2006) and animals (Jarne and Auld, 2006; Escobar et al. 2011) show an excess of very high and very low rates. However, there is increasing evidence for evolutionary transitions between these states. For example, comparative studies in several angiosperm groups revealed that selfing species often derived recently from outcrossing lineages (e.g., Escobar et al. 2010 in the Triticae; Goldberg et al. 2010). These transitions may occur rapidly, as documented in the selfer *Capsella rubella* which evolved less than 50,000 years ago from the self-incompatible *Capsella grandiflora* (Brandvain et al. 2013, Foxe et al. 2009, Guo et al, 2009). A possible scenario for these transitions is that facultative selfing may sometimes take place in an initially outbred population with the potential to quickly purge ID, which eventually favors high selfing rates (Jarne and Charlesworth 1993; Byers and Waller, 1999). Conversely, occasional outcrossing is unlikely to occur in a low-ID selfing population for a sufficient time and at a rate high enough for ID to re-accumulate through *de novo* mutations.

If facultative selfing triggers evolutionary transitions towards preferential selfing, environmental factors may play an important role. The reproductive assurance hypothesis predicts that shortage of pollinators or of mating partners may favor self-fertilization (Baker 1967; Cheptou 2012). The relatively high fraction of intermediate selfing rates observed in flowering plants might reflect the effect of adverse pollination and/or fertilization conditions in otherwise preferentially outcrossing species (Goodwillie et al., 2005). For example, plants may self-fertilize at the end of the reproductive season the flowers that failed to be pollinated during the season (delayed selfing; e.g. Kalisz et al. 1999). A behavior akin to delayed selfing has been described in freshwater snails (Escobar et al. 2011; Tsitrone et al. 2003b), cestodes (Schjørring 2004) and free-living flatworms (Ramm et al. 2012). Isolated individuals delay their first reproduction, compared to grouped individuals, before eventually self-fertilizing their eggs. The difference in age at first reproduction between isolated (self-fertilizing) and grouped (outcrossing) individuals, known as the waiting time, provides a quantitative measure of the extent to which individuals are reluctant to self-fertilize (Tsitrone et al. 2003a).

Transitions from outcrossing to selfing might also be accompanied by an evolution towards characteristic phenotypic traits known as the selfing syndrome (see Sicart and Lenhard 2011 in plants, Escobar et al. 2010 in animals). Selfing organisms are indeed expected to invest far less in the male than in the female function as a result of relaxed sexual selection (Charlesworth and Charlesworth 1981; Charnov 1982). For example, selfing angiosperm species often show a reduced male allocation in terms of inconspicuous flowers and low pollen production (Barrett 2003). Similarly, selfing animals produce few sperm (Charnov 1982; Johnston et al. 1998), and invest little in mate acquisition (Jarne et al. 2010). A decrease in male *vs*. female allocation is therefore expected during or after the transition towards selfing.

To summarize the arguments above, a scenario of an evolutionary transition from outcrossing to selfing is provided in Fig. S1. Starting from an outcrossing population with high ID, strong resistance to selfing (*i.e.* traits preventing selfing, including the waiting time) and equal male-to-female sex allocation, we end up with a selfing population exhibiting low ID, limited resistance to selfing and low investment in the male function. Although all steps in this scenario seem plausible in theory, the joint evolution of these traits has never been observed directly. This is the aim of this study in which we use an experimental evolution approach. Obviously experimental evolution is not likely to reproduce in a reasonable time the characteristics of natural transitions, both because the natural conditions cannot be recreated and because the timescale of natural evolution is probably much longer. However it may indicate whether populations start to evolve in the expected direction under laboratory conditions representative of the scenario from Fig. S1. Experimental evolution has been used only in a few cases to investigate the evolution of mating systems in hermaphrodites. For example, Bodbyl and Kelly (2011) observed a reduction in anther-stigma distance in less than five generations of selfing when pollinators were excluded in the plant *Mimulus guttatus*. In animals, experimental evolution was used in the androdioecious nematode *Caenorhabditis elegans*. Morran et al. (2009) showed that an elevated mutation rate and the exposure to novel selective environments favor an increase in both male production and outcrossing rates in inbred lines initially devoid of genetic variation, and Chelo et al. (2014) reported that androdioecy favors a faster purge of the mutation load compared to dioecy in genetically variable populations. However natural selfing rates are extremely high in *Caenorhabditis elegans*. To our knowledge, experimental evolution has never been used to investigate the transition from outcrossing to selfing in an initially outcrossing hermaphroditic animal.

We here use an experimental evolution approach in freshwater pulmonate snails to test the above-mentioned scenario (see Fig. S1). Transitions from outcrossing to selfing are

suspected in several genera of this group (Jarne et al. 2010). Species exhibit either high (>0.75) or low (<0.25) selfing rates and high outcrossing rates are associated with high inbreeding depression and long waiting times (Escobar et al. 2011). More detailed studies in *P. acuta*, a preferential outcrosser, indicated heritable variation in both waiting time and ID, within and among populations as well as a positive genetic correlation between these two traits (Escobar et al. 2009). We constructed selection lines of *P. acuta* that were maintained under conditions of either mate limitation, or of free access to mates, for 17 and 23 generations respectively. Experiments were conducted in order to test whether mate limitation leads to (i) decreased waiting time, thus limiting the propensity to outcross, (ii) purged ID, and (iii) decreased male allocation, resulting in lower male fitness, with reallocation towards the female function.

Materials and methods

Species studied and experimental conditions

The gastropod *P. acuta* (Hygrophila) has a cosmopolitan distribution and occupies various types of freshwater habitats (Dillon et al. 2002; Bousset et al. 2014). Individuals lay egg capsules, typically containing a few tens of eggs. Hatching occurs within 7-10 days after egg-laying, and sexual maturity is reached at six to eight weeks at 25°C under laboratory conditions. This simultaneous hermaphrodite can reproduce through both self- and cross-fertilization, although it preferentially outcrosses (Henry et al. 2005; David et al. 2007). Selfing occurs when individuals cannot find mates, and is associated with strong ID (Jarne et al. 2000; Escobar et al. 2008; Janicke et al. 2013) and a delay in first reproduction (the waiting time; Tsitrone et al. 2003a). Mating is unilateral with one individual acting as male while the other acts as female. It involves a simple behavioural sequence (Wethington and

Dillon 1996; Facon et al. 2006; Jarne et al. 2010) during which the male-acting individual crawls on its partner shell, and introduces its phallus into the partner's gonopore. The whole sequence lasts for several tens of seconds to 20–30 min and can easily be observed (Wethington and Dillon 1996; Facon et al. 2007; Pélissié et al. 2014). *Physa acuta* is an appropriate species for our study because (i) selfing occurs facultatively, (ii) natural populations experience episodes of low density during which opportunities of cross-fertilization are severely reduced (Henry et al. 2005), and (iii) it has a short generation time.

During the whole experiment, including the construction of experimental lines, snails were maintained at 25°C in groundwater from a nearby well under a 12:12 photoperiod, and fed *ad libitum* with boiled lettuce twice a week. Water was changed once or twice a week, and individuals were kept most of the time in 75-mL plastic boxes.

Construction of experimental evolution lines

We generated four experimental lines of *P. acuta* from a single base population obtained by mixing genotypes that have been collected from several natural populations (Fig. 1). We sampled at random 62 juvenile (virgin) individuals in November 2007 in 10 populations (6.2 ± 1.8 SD individuals per population) around Montpellier, France (Supporting Information Table S1), some of which have been studied in previous work (Bousset et al. 2004; Henry et al. 2005; Escobar et al. 2008). Inter-population pair crosses were conducted over two generations to produce 53 full-sib families and 941 F2 individuals (approximately 20 offspring per family) that were subsequently pooled together. The crosses were planned in such a way that each F2 had grandparents from four different populations, and that all populations contributed approximately equally to the gene pool (0.10 ± 0.04 SD). These controlled inter-population crosses over two generations reduced linkage disequilibrium and ensured a wide genetic basis to the base population (G0).

Two line types, C (control) and S (frequent selfing), were derived from the base population, each with two independent replicates (C1, C2; S1, S2). Each replicate evolved at a population size of 70-80 adults per generation. Each new generation was initiated by pooling a large number of hatchlings together in a large aquarium for a week. One hundred and thirty-five of them were then randomly sampled and placed in boxes (three juveniles per box) for a week. Ninety of them were then randomly chosen and isolated for two more weeks, *i.e.* the required time to reach sexual maturity. At this stage, the experimental conditions for C and S lines started to differ.

In the C lines, all (90) mature individuals were grouped in a large aquarium (3L) in which they freely mated for a week (mass-mating). As virgin individuals are very eager to mate, all individuals were expected to repeatedly copulate as both male and female (Pélissié et al. 2014). These experimental conditions mimicked a high-density natural population and selection acted on both the male and female fitness components. After mass-mating, individuals were isolated and their egg capsules collected over a week. All living juveniles were collected after hatching (two weeks after isolation), pooled in an aquarium and a new cycle was initiated. Note that the failure to get juveniles from some parents explains that population size was 70-80 rather than 90.

Adults from the S lines followed a different cycle, with alternating regimes in odd- and even-numbered generations. In odd-numbered generations, 90 adults remained isolated until at least 2/3 of them had laid selfed eggs (enforced selfing) - this usually required collecting capsules over two successive weeks. In even-numbered generations, 90 randomly-chosen individuals were paired for a week (random pair-mating) and capsules were monitored until hatching. In both situations, juveniles were pooled, as in the C lines, to initiate the new generation. We did not enforce selfing each generation, based on previous experience suggesting that too many lines would have been lost given the large ID in *P. acuta* (Jarne et

al. 2000). However random pair-mating substantially reduces the opportunity of mate encounter (*i.e.* still mimics low population density) while limiting the accumulation of genotypic disequilibria and line loss. The selection regime on the female function was similar in the C and S lines since the contribution of each mother to the next generation was proportional to their offspring production in both cases. The selection on the male function was much weaker in the S than in the C lines, as S individuals were never exposed to pre- and post-copulatory male-male competition.

For both selection regimes, we set a minimum target population size of 68 mothers contributing to the next generation per replicate. When mortality reduced the pool of mothers to a lower value, we replaced missing individuals with individuals from the previous generation of the same replicate, maintained in a backup tank. This slowed down the evolutionary processes but limited the undesired consequences of population bottlenecks. In addition, generations were shorter in the C than in the S lines. This is due to the fact that isolated individuals initiate reproduction later than paired individuals because self-fertilization is typically delayed by the waiting time. The number of generations of experimental evolution was therefore lower in the S lines (17 including eight of selfing) than in the C lines (23) when the experiment was initiated (Fig. 1).

Albino lines used in crosses

All experimental individuals from the S and C lines were wild-type pigmented snails. We created albino lines in order to easily estimate the male reproductive success of these individuals. Albinism is common among freshwater snails (Jarne et al. 1993), and has previously been used for controlled crosses in *P. acuta* (Dillon and Wethington 1992). Albino individuals, be they adult or hatchlings, are easily recognized by a pale mantle and a lack of eye pigmentation. Albinism has a simple genetic basis, with a dominant pigmented

allele and a recessive albino allele at a single locus. In mating groups of homozygous individuals, the number of pigmented offspring laid by albino mothers provides an estimate of the siring success of pigmented fathers.

Albinism appeared in a selfed brood from the S2 line during the first generations of selection. We multiplied the albino individuals for two generations and then introgressed the albino allele into the C1 and C2 populations. At the time of the experimental assays, we had two albino populations ($C1_{alb}$ and $C2_{alb}$), each maintained at N > 100 individuals. Each population had ¾ of its genome deriving from the corresponding C population and ¼ from the initial albino brood. Individuals from $C1_{alb}$ and $C2_{alb}$ were then submitted to two generations of random mating prior to the assay. We can therefore roughly consider $C1_{alb}$ and $C2_{alb}$ individuals as outbred individuals deriving from the early generations of the C1 and C2 lines. The behavioral and life-history traits of these albino lines have been shown to be similar to those of wild-type offspring of individuals from natural populations (Janicke et al. 2014).

Selection response assay

Around 60 outcrossed virgin individuals were randomly chosen per replicate of the S and C lines (Fig. 1) to serve as parents of the focal individuals. Forty parents were randomly paired, and the 20 remaining parents were kept isolated. Egg capsules were collected and the hatched juveniles were isolated around three weeks after egg laying (*i.e.* well before sexual maturity). Traits were measured on these (focal) snails and their offspring (see below). The focals were either outbred when deriving from paired parents, or selfed when deriving from isolated parents. Additionally we kept track of family (*i.e.* maternal) identity of each focal. Outbred focals were then split into three assay treatments: (i) isolated outbreds (IO) remained isolated which enforces selfing. (ii) Pair-mated outbreds (PMO) were paired three times a week for three hours with a sexually mature mate from the same replicate, providing the

opportunity to outcross, and were otherwise isolated. Such a procedure ensures outcrossing in focals while avoiding density effects on fitness (Tsitrone et al. 2003a; Escobar et al. 2011). This treatment started before sexual maturity and lasted until eggs were laid, in order to record the age at first reproduction in conditions of constant mate availability. (iii) Outbreds mated with albinos (MAO) were isolated up to 45 days of age, before being grouped for 48 h with two virgin albino individuals of the same age, in order to record male and female reproductive performances of focals at a constant age. The albinos were marked with a paint dot in order to follow individual status during the mating interactions. Previous work has shown that paint marks do not affect fitness traits (Henry and Jarne 2007). The three individuals were then isolated and their egg capsules collected over seven days. Under this MAO treatment, reproduction was expected to occur by outcrossing with male-male competition. From the focal viewpoint, each albino served both as a female mate and as a male competitor. The selfed focals experienced a single treatment, *i.e.* mating with two albino individuals (MAS; similar to MAO). The number of focals were 109 (IO), 109 (PMO), 80 (MAO) and 90 (MAS), approximately equally shared among the four lines. Focal offspring were counted during 15 days after egg-laying.

Traits measured

We measured several traits in focals and their offspring in the four treatments (Fig. 1). Note that not all traits and treatments were used to address each of the three questions mentioned in Introduction. The first question is whether snails are less reluctant to self-fertilize in the S than in the C lines. This was evaluated using the waiting time, *i.e.* the difference in age at first reproduction between isolated and paired snails. We also considered its equivalent in terms of body mass, the added weight (*i.e.* the difference in body mass at first reproduction between isolated and paired snails), which represents the expected reward of

waiting in terms of future egg production, since reproductive output and weight are correlated in *P. acuta* (Tsitrone et al. 2003b). We measured the age and body weight at first reproduction of outbred focals from the four lines that were either isolated (IO treatment), or paired (PMO). The MAO and MAS treatments were not used because the first mating opportunity was provided after sexual maturity.

The second question is whether ID has been purged in the S lines. ID is the difference in fitness between outcrossed and selfed individuals and can be measured on both juvenile and adult traits. To estimate ID on juvenile survival, we counted the egg number laid by focals from the IO, PMO and MAO treatments over four days after the first reproduction (sometimes more, to get enough eggs). Juvenile survival was estimated as the number of surviving hatchlings 15 days after egg collection (this includes hatching success and early survival). Comparing the IO *vs*. PMO or MAO treatments allowed estimating ID on this trait. The MAS treatment was not considered since the focal's mothers were inbred.

ID was also estimated on adult traits. We compared body weight at 45 days between the MAO and MAS treatments, *i.e.* at the time of first mate encounter. The IO and PMO treatments were not included in the comparison because they differ in reproductive history. We also recorded traits related to mating interactions and reproductive success in the MAO and MAS focals. Mating-behavior traits were measured at 45 days through 45-minute observation of mating groups (one pigmented focal plus two individually-tagged albinos, one from each albino line) right after group formation using the method described in Pelissié et al. (2012). For each focal, we recorded the number of copulations in both sex roles. Male sexual activity was quantified by the time spent in male position (*i.e.* on top of the partner's shell, whether copulation was occurring or not) and by copulation duration (*i.e.* phallus actually inserted under the partner's shell). Female copulations were similarly monitored when the focals played the female role. The three individuals were then maintained together for 48

hours before being isolated. Their egg capsules were collected over three days or more. Survival was estimated 15 days later together with the fraction of pigmented juveniles among the offspring of albino partners.

We computed female reproduction (offspring per day) as the product of three (multiplicative) components: probability to lay eggs, fecundity of laying individuals (eggs per day) and survival of maternal offspring. Similarly we estimated male reproduction (sired offspring per day and mate) as the product of the daily production of juveniles per female mate, the probability to sire offspring laid per female mates, and paternity share (proportion of offspring sired by the focal individual). Comparing the MAO and MAS treatments produced estimates of ID for female and male reproduction.

The third question was whether a more female-biased allocation had evolved in the S lines. It was addressed using the male and female traits mentioned above (reproductive and behavioural traits; Table 1), related to mating interactions and reproduction, and comparing the S and C lines, independently of the MAO or MAS treatments. These comparisons included measures of both sex-specific investment (*i.e.* egg-laying activity and female copulatory behaviour for the female function; male copulatory behaviour for the male function) and sex-specific fitness (maternal and paternal offspring).

Additional experiment for ID on juvenile survival

Our analyses revealed large variation in juvenile survival among broods leading to an imprecise estimate of inbreeding depression. In order to obtain a better estimate, we ran a separate experiment including a larger number of individuals and focusing exclusively on juvenile survival. We sampled outbred virgin juveniles from each of the four lines one generation after the main experiment (61.5 per line on average, issued from egg capsules laid in an aquarium hosting 90 parents) and isolated them until maturity. Half were pooled

together for three days (mass-mating within line) before being isolated again, and the other half were isolated. Egg capsules were collected over a week, and egg numbers and juvenile survival were estimated as previously. Comparing isolated and grouped individuals yielded a second estimate of ID on juvenile survival.

Statistical analyses

All analyses were based on generalized linear mixed models (GLMs). Fixed effects included treatment, line type (C *vs*. S) and their interaction. Replicate within line type, family within replicate and their interactions with treatment were included as random effects. As the experiment lasted over three weeks, we added a block effect (fixed) to account for possible changes in environmental conditions. The experiment comprised three blocks each containing all individuals born a given week for all traits, except mating behaviour traits for which time constraints imposed to make two observation blocks per week (first half, second half). Depending on the trait studied, the analyses included or not body weight as a covariate, and used either Gaussian (most traits), Poisson (copulation number) or binomial (survival, probability to copulate, to lay eggs, or to sire offspring) error distributions. For non-Gaussian variables we corrected for overdispersion when necessary by adding observation number as a random factor (see Browne et al. 2005; Elston et al. 2001). We first simplified models by eliminating as many non-significant random terms as possible. The block effect was then tested for, and removed when non-significant. The effects of interest (treatment, line type and their interaction) were finally evaluated using model simplification. When the interaction term was significant we tested the line type effect within each treatment separately, and vice versa. For male fitness traits, we had two estimates per focal since each focal was mated with two albino partners (MAO and MAS treatments). "Focal individual" was added to the models as an additional random factor to account for the correlation between the two measures for a

given focal. For each brood we included the body weight of the mother, her line ($C1_{alb}$ or $C2_{alb}$) and the body weight of the competitor (*i.e.* the other albino individual involved in the assay). These covariates were included in the initial models, and discarded when non-significant.

All comparisons were conducted using chi-square likelihood-ratio tests. P-values for random effects and their interaction with fixed effects were corrected (as advised by Zuur et al. 2009). For Gaussian-distributed traits we computed likelihood ratios using restricted maximum likelihood for random terms and maximum likelihood for fixed effects. All analyses were performed with R version 3.0.0 packages lme4 (Bates et al. 2013) and nlme (Pinheiro et al. 2013).

Expectations

We summarized in Table 1 our expectations on the reluctance to self-fertilize, ID and sex allocation, formalized above as three questions, and based on the arguments exposed in Introduction (see also Supporting Information Fig. S1). It is worth noting that the purging of ID is not expected to be identical for all traits. First, ID on juvenile survival is probably more prone to purging because early-expressed recessive lethals or semi-lethals are expected to act early in life while adult traits are probably mostly dependent on slightly deleterious alleles (Lande et al. 1994). ID is indeed much stronger in the early stages of life in *P. acuta* (Jarne et al. 2000). Second, we do not expect ID on male reproductive traits to be purged in the S lines because selection on the male function is relaxed (no male-male competition). This is also true for female copulatory traits (copulation number and time being mounted) indicating the attractiveness of these individuals as potential female mates – here again selection is relaxed in S lines given the absence of mate competition. Note however that ID for these traits could

be indirectly purged as a result of being genetically correlated with survival, growth or fecundity.

Results

Traits indicative of the reluctance to self-fertilize eggs

The selection regime had a significant effect on the waiting time as indicated by a significant interaction between line type and treatment (Tables 2 and S2, Fig. 2). Specifically, the age at first reproduction was delayed by seven days in the S lines, while it was delayed by 17 days in the C lines. The line type effect was not significant in the PMO treatment, so that the difference in age at first reproduction between the C and S lines is due only to the IO treatment (Table 2). The same results were obtained on body weight at first reproduction. C individuals raised in isolation were nearly twice as heavy as their paired congeners, when they started to lay eggs, while this difference was only around 30% in the S lines (Tables 2 and S2, Fig. 2).

Inbreeding depression: juvenile survival

Juvenile survival was first compared between the IO, PMO and MAO treatments. Treatment did not affect juvenile survival in the same way depending on line type (Tables 2 and S2). Indeed, juvenile survival did not vary significantly across treatments in the S lines while it did in the C lines (Table 2). In the latter, survival was minimal in inbred offspring (IO), intermediate in offspring of pair-crosses within line (PMO) and maximal in outbred offspring obtained by crossing with external individuals (MAO) (Fig. 3a, Table S2). As a result differences in juvenile survival between C and S lines were significant when juveniles

were inbred but not when they were outbred (Table 2). ID estimates (obtained by comparing PMO and IO) were clearly higher in the C lines (Table 2), but even in these lines the difference between PMO and IO was not significant (post-hoc test) due to a large variance in survival, especially in the PMO treatment. The additional experiment on juvenile survival confirmed that ID was much lower in the S (0.10 +/- 0.11) than in the C (0.50+/-0.10) lines with a significant drop in survival upon inbreeding in C, but not in S, lines (Fig. 3b, Table 2).

Inbreeding depression: adult traits

In the MAO and MAS treatments, body weight at 45 days (before mating) differed between line types as individuals from S lines were smaller than those from C lines (Table S2; Fig. 4). ID was significant and did not differ between the two line types (Table 2). The values of the three components of female reproduction (probability to lay eggs, fecundity of laying individuals and survival of maternal offspring) are reported in Fig. 4 and Table S2. On average 17% of individuals did not lay eggs and this proportion did not differ significantly between line types or treatments (Table 2). The fecundity of fertile individuals was moderately but significantly affected by treatment (ID) and the difference between C and S lines was not significant (Table 2). Female fecundity was strongly dependent on body weight (Table 2) though incorporating the latter as a covariate did not change the observed patterns. Offspring survival was not significantly affected by either maternal origin (line type), or maternal inbreeding, although offspring from inbred mothers tended to survive less (Table 2). This tendency disappeared once maternal size was added to the model (Table 2). Results on female reproduction therefore mostly reflected the patterns observed for female fecundity, *i.e.* moderate ID and no difference between line types (Table 2).

The values of the three components of male reproduction (daily production of juveniles per female mate, probability to sire offspring laid by female mates, and paternity

share) are reported in Fig. 4 and Table S2. Inbreeding status and line type of focals did not significantly affect the production of offspring by their female mates. We note however that inbreeding had a large negative effect on both the probability to sire offspring and paternity share (Table 2). In particular, we observed a significant line type by treatment interaction for paternity share, as inbreeding depression was very large in S lines (0.56+/-0.07), but moderate in C lines (0.21+/-0.10). This difference mainly emerged from a much lower paternity share in inbred (MAS) individuals in S lines than in C lines, while paternity share in outbred individuals (MAO) was similar in the two line types (Table 2). Similar patterns were obtained for male reproduction as a whole. Including focal body weight as a covariate did not qualitatively modify the results.

Copulation behavior

The values of the three behavioral traits recorded are reported in Fig. 5 and Table S2. Most individuals interacted with mates as females (80%) and/or as males (55%), and we observed zero to three copulations in both roles. However we observed no significant effect of treatment, line type or interaction on any of these variables, whether or not we corrected for body size (Table 2).

Discussion

High selfing rates in hermaphroditic species are associated with a suite of traits, including a variety of characters that tend to facilitate selfing (e.g., reduced anther-stigma distance in plants and reduced waiting time in snails), low inbreeding depression, and a female-biased sex allocation – collectively known as the selfing syndrome. This has been observed in both plants and animals (Cruden, 1977; Barrett 2003; Escobar et al. 2011) but an

experimental evolution approach has rarely been undertaken (but see Chelo et al. 2014; Morran et al. 2009). We conducted such an approach in the freshwater snail *Physa acuta* in order to evaluate whether this syndrome can evolve, and at what pace.

A decreased waiting time

We first focused on traits indicative of the reluctance to self-fertilize, *i.e.* on the age and weight at first reproduction and on the waiting time. Previous studies based on a large number of populations have reported a waiting time of 15 to 20 days – the age at first reproduction is around 30-40 days in *P. acuta* (Tsitrone et al. 2003b; Escobar et al. 2009). A waiting time has also been reported in other outcrossing animals (Escobar et al. 2011). The waiting time has been interpreted as a mechanism to cope with temporal variation in mate availability in heterogeneous environments (Tsitrone et al. 2003a), for example in case of fluctuating population density due to dessication-refill cycles in ponds inhabited by freshwater snails. As expected, we observed a lower waiting time in S lines when compared to C lines, with a decrease of about 60% in 17 generations. The age at first reproduction of isolated individuals, but not that of paired ones, evolved in the S lines. This is consistent with the much larger genetic variance in the former, and also with what is observed in natural populations of *P. acuta* (Escobar et al. 2007, 2009). Heritability estimates of the age at first reproduction in isolation obtained in previous studies (between 0.3 and 0.46 in Escobar et al. 2007, 2009) can be used to estimate the strength of selection required to explain its evolution, and therefore that of the waiting time, in the S lines. Assuming that selection occurred only when the trait was expressed (*i.e.* selfing generations) it amounted to the elimination of those 8 to 13% individuals with the highest trait value per generation (in truncation selection equivalents; Falconer and Mackay 2009). Interestingly, we obtained the same results for the

weight at first reproduction than for the age at first reproduction, as already observed by Tsitrone et al. (2003b). This is not surprising since age and weight (shell size) are correlated in *P. acuta*, but opens the way to possible reallocation between current reproduction (egg-laying) and future reproduction (in the form of stored biomass), an important feature of the evolution of the waiting time (Tsitrone et al. 2003b). How the reluctance to self-fertilize may evolve over several generations of selection has been evaluated in a limited number of species. Bodbyl and Kelly (2011) observed an increased production of autonomous seeds and a decreased anther-stigma distance in *M. guttatus* when pollinators are absent. Further similar studies are required to confirm that the mating system can evolve quickly as a result of mate or pollinator limitation, and possibly extend them to the response to long-term environmental changes (see Thomann et al. 2015 for an example).

Inbreeding depression is purged, essentially in the early stages of life

ID is usually high in natural populations of *P. acuta* (Jarne et al. 2000; Escobar et al. 2007, 2009), and the strong ID on early survival observed in C lines is in the range of previous studies. ID in the S lines is comparatively significantly milder. It results from an increase in the survival of inbred offspring, rather than a decrease in that of outbred offspring, suggesting that purging of deleterious mutations occurred in the S lines, rather than their fixation (which would have resulted in the opposite pattern). Purging through enforced selfing (or sib-mating) over several generations has already been experimentally tested in a few experiments, with mixed, though generally positive, results (e.g., Barrett and Charlesworth 1991; Carr and Dudash 1997; review in Crnokrak and Barrett 2002). However our S lines differ from most of these studies by mimicking populations with frequent, though not continuous selfing. Our conditions are probably more representative of natural settings in which selfing may occur as an occasional strategy of reproductive assurance in the face of

increased uncertainty in pollinator and/or mate availability. Moreover the opportunity for competition between selfed individuals harboring different mutation loads is high in the S lines as competition occurs among unrelated broods, and not within sibships. On the whole, our experiment illustrates that rapid purging can occur in conditions mimicking environmentally–induced variation in outcrossing opportunities, possibly in an even more efficient fashion than in classical artificial settings enforcing extreme inbreeding and population substructure. Additionally the loss of genetic variability is slowed down in our protocol by recombination during outcrossing events once every two generations, and purging presumably occurred much more through selection than through genetic drift (Glémin 2003).

ID on adult traits was much more limited than on juvenile survival – we observed very limited effects on size and female fecundity, and even larger ID in the S than in the C lines for male fecundity (evaluated under competitive conditions). Deleterious mutations acting on male fecundity were indeed not expected to be purged, since male-male competition is relaxed in the S lines. ID on male fitness was high in both types of lines, as already shown in the single previous study evaluating this component of ID in *P. acuta* (Janicke et al. 2013). Interestingly, although female reproductive traits were under selection in the S lines, they did not undergo significant purging either. This result, compared to the strong effects observed for early survival, suggests that different genetic architectures are involved, as already suggested in the plant literature (Husband and Schemske 1996). Early components of fitness are affected by a few loci with large effects which can be quickly purged, while large number of loci with small effects (which affect all traits) are not expected to undergo strong changes in frequency in the course of a few generations (Lande et al. 1994; Husband and Schemske 1996; Chelo et al. 2014).

Even if restricted to juvenile traits, genetic purging should favor a switch towards self-fertilization in case of mate limitation (Tsitrone et al. 2003a). It might be involved in the

S lines in a coevolutionary loop with the reduction in the waiting time favoring the evolution of selfing. Escobar et al. (2009) detected a positive genetic covariance between ID and waiting time both within and among natural populations of *P. acuta,* which suggests that such a loop can operate in nature. The next question is whether purging can reduce ID up to a point when preferential (prior) selfing becomes advantageous, even when mates (or pollen) are available. Lifetime ID in *P. acuta* can be roughly estimated by combining results on juvenile survival and adult fecundity ($1\text{-}ID_{total} = (1\text{-}ID_{juvenile})*(1\text{-}ID_{fecundity})$), since most juveniles surviving the first 15 days thenafter reproduced as adults. Using estimates from Table 2, we obtain a total ID of 0.29 and 0.74 in S lines for female and male fitness respectively, and the corresponding values are 0.60 and 0.74 in the C lines. The latter both exceed 0.5, the critical value of ID that favors preferential outcrossing in genetical models (neglecting sperm discounting). This threshold was crossed in the lines exposed to mate limitation, but only for the female function – the high ID on the male function is probably due to relaxed selection on the male function. Interestingly, this change in ID is expected to be sufficient to leave the zone of evolutionary stability of pure outcrossing in theoretical models accounting for both male and female fitness. The maintenance of a high male ID suggests that the S lines could be invaded by mutants with an intermediate rate of prior selfing, leading to a mixed-mating system (Rausher and Chang 1999). Finally we note that situations of mate limitation may also lead to population bottlenecks in natural conditions, a factor favoring the further decrease of ID (Kirkpatrick and Jarne 2000) and therefore of higher selfing rates.

No evolution of sex allocation

Theory predicts that the equilibrium male / female allocation should be much lower in selfers than in outcrossers, assuming a sex allocation trade-off (Charlesworth and Charlesworth 1981; Charnov 1982). This has indeed been observed in selfing plants that show

both a reduced pollen / ovule ratio and inconspicuous flowers (Cruden 1977). Much less empirical evidence has been accumulated in animals, but highly selfing species tend to produce less sperm, relative to eggs, in hermaphroditic bivalves (Johnson et al. 1998). In freshwater snails, investment into the male function can partly be captured through male copulatory activity (Jänicke et al. 2013; Auld et al. 2014) while most of the energetic investment in the female function is probably represented by egg production. Predominantly selfing species are expected to copulate less often relative to egg-laying activity than predominantly outcrossing ones, as examplified by the comparison between the selfing species *Biomphalaria pfeifferi* and its outcrossing relative *Biomphalaria glabrata* (Tian-Bi et al. 2008). We note that both theory and data apply to (equilibrium) situations in which selfing has been the main reproductive system for a long time, but little is known on how and at what speed the transition in resource allocation occurs. An important question is whether, in an initially outcrossing species, conditions that enforce selfing also select for a more female biased sex allocation. Thomann et al. (2013) pointed out that pollinator limitation in angiosperms can either result in increased flower attractiveness in order to maintain high outcrossing rate, or lead to prior selfing and inconspicuous flowers when pollen limitation is so strong that outcrossing becomes too costly. By analogy, we might expect that snail populations experiencing mate limitation either invest more into mate search, mating attempts and/or copulations, or to invest less into these traits when the probability of encounter drops sharply. The conditions of our experimental evolution lines rather mimic the latter situation, with relaxed selection on the male function in the S lines. We therefore expected a reduction in male allocation in the S lines when compared to the C lines.

We did not detect obvious changes in male and female sex allocation. Moreover, although outbred individuals from the S and C lines had similar male and female fitness (in offspring/day), inbred individuals in the S lines had lower paternity share in competitive

conditions than their counterparts from the C lines. As a consequence, ID on these traits was larger in the S than in the C lines. This suggests that changes in male fitness in S lines compared to C lines rely on predominantly recessive alleles, the effect of which is enhanced by the increased homozygosity following selfing. Classic arguments on the evolution of sex allocation rely on the assumption that alleles that reduce male fitness tend to increase female fitness (Schärer 2009). We do not believe however that such alleles were involved here because we saw no increase in female fitness in S individuals, even in inbred ones, although the latter had lower male fitness. Therefore, we rather interpret the decreased male fitness in S lines as the result of an increase in the frequency of some male-specific recessive deleterious alleles initially rare in the base population, following relaxed selection on the male function. Note that this relaxation is only partial for copulation traits (as individuals reproduce in pairs every second generation in the S lines) while it is stronger for traits involved in male-male sexual competition for outcrossing (as S individuals never had competitors). This may explain why paternity share, but not copulatory traits, seems to have evolved under our competitive conditions. Deleterious mutations are usually recessive (see Agrawal and Whitlock 2011; Manna et al. 2011), so that their effect should be detected mainly in inbred individuals.

Our results on the male function are reminiscent of those obtained in several experimental evolution studies in separate-sex organisms, in which male-male competition was similarly relaxed through enforced monogamy. In such experiments male investment and fitness traits (e.g., testis and body size, sperm production, paternity share) generally decreased without any obvious re-allocation to other traits, as if they were free to accumulate deleterious mutations (Hosken and Ward 2001; Pitnick et al. 2001; Simmons and Garcia-Gonzalez 2008; Firman and Simmons 2009). In a recent study this loss was accompanied by a parallel loss in female fitness suggesting positive genetic correlations between male and female fitness (Lumley et al. 2015). However in hermaphrodites, unlike in gonochorists, reallocation could

directly occur from the male to the female function within an individual (Schärer 2009). As we did not detect reallocation, we hypothesize that a significant fraction of the standing variation in male fitness traits in our snails relies on low-frequency mutations that are deleterious to the male function and not particularly beneficial to the female function. During the first generations of relaxed selection on male-male competition, such mutations may start to accumulate through genetic drift. More generations are now needed in our experiment to test whether (i) this accumulation will get to the point at which the male traits of outbred also decrease in a detectable way, and (ii) female fitness starts to increase as expected if a few sex-antagonistic mutations were segregating (even if they contributed a small fraction of the initial genetic variance).

Conclusion

Experimental evolution has already been used in *C. elegans* to assess how predominant self-fertilization facilitates short-term purging but impairs long-term adaptation (Morran et al. 2009, Carvalho et al. 2014). However we are not aware of experiments in which selfing was enforced in a preferential outcrosser in order to analyse the consequences on fitness and reproductive traits. On the whole, we observed that both ID and the waiting time evolved in the direction expected when a population is undergoing an evolution from outcrossing to selfing. We showed that populations can adapt to the lack of partners (or pollinators) by changing their reproductive traits (reduced waiting time) in a few generations, paving the way to an increased selfing rate. Interestingly, these conditions also have profound consequences on the mutation load, as the purging of deleterious mutations (at least those acting early in life) reduced lifetime ID enough to leave the zone of evolutionary stability of preferential outcrossing. However relaxed selection on the male function under constrained selfing did not appear to result in reallocation to fitness, but rather in the accumulation of

male-specific deleterious mutations. More generations are required to investigate how this system will evolve on a longer time scale, especially whether (i) ID on late components of fitness is eventually purged, (ii) the waiting time decreases to zero, (iii) reallocation to the female function occurs, and (iv) spontaneous selfing become detectable and copulation activity reduced even in free-mating conditions. Other traits, especially related to the structure of the reproductive apparatus (e.g., a simplified prostate; Jarne et al. 2010), are also expected to evolve, and this might be evaluated in further studies. Moreover similar experiments should ideally be performed in other hermaphroditic organisms.

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Figure legends

Figure 1. Experimental protocol. The experimental evolution lines (selfing and control) were run for 23 (G23) and 17 (G17) generations respectively before initiating the experimental assay represented in the middle to lower parts of the figure. Individuals from both the outcrossing and selfing lines were either paired, or outcrossed, to generate focal individuals. Outbred focals were isolated (IO treatment), paired before sexual maturity (PMO), or isolated up to the age of 45 days, before being paired with two albino partners (MAO). Selfed focals were isolated (MAS). The traits monitored on focals and their offspring (focals offspring) are indicated on the right. More details in text. N is the number of individuals.

Figure 2. (A) Age at first reproduction (AFR) and (B) body weight at first reproduction (WFR) in individuals from C and S lines in the PMO (pair-mating) and IO (isolated) treatments. Data are presented as means +/- SEM, averaged over experimental blocks. The waiting time (WT) and added growth (ΔW), which measure the reluctance to engage in self-fertilization, are indicated with arrows.

Figure 3. Juvenile survival in inbred and outbred offspring of C and S lines. (A) In the main (first) experiment for the MAO (mating with two albino partners), PMO (pair-mating) and IO (isolated) treatments. (B) In the extra (second) experiment: outbred mothers produced either outcrossed, or selfed offspring. Data are presented as means +/- SEM, averaged over experimental blocks.

Figure 4. Body weight and female and male reproductive traits of focals and focals' offspring (means +/-SEM; corrected for both block effects and covariates describing variation

in mate origin and quality) in outcrossed (MAO) and selfed (MAS) individuals from different line types. Female and male reproduction (right panels) are the products of the three components in the first three panels on the relevant row. Graph legends (x axis) are identical to that of the upper graph (body weight).

Figure 5. Traits describing female and male mating behavior of focals measured during a 45-mn encounter with two albino mates (means +/-SEM; corrected for both block effects and covariates describing variation in mate origin and quality) in outcrossed (MAO) and selfed (MAS) individuals from different line types. Graph legends are identical to that of the upper left graph (probability of being mounted)

Table 1. Expectations based on the transition scenario from Figure 1. The waiting time (WT) and inbreeding depression (ID) are by construction derived from the comparison of two treatments, and are therefore tested as interactions between treatment and line type (S *versus* C). In contrast reproductive allocation is estimated within treatments and we focus on the main effects of line type. The expectations on these three parameters are given in the last column – when comparing the S and C lines. All traits were measured on focal individuals (see Fig. 2) except juvenile survival and paternity success which were estimated in their offspring. Traits involving the female function are overlaid in gray. Note that ID is not expected to be purged for some adult traits in the S lines because of relaxed selection (see Text).

Questions	**Parameters**	**Traits**	**Treatments**	**Expectation (S *vs*. C)**
1 - Does reduced reluctance to selfing evolve under mating constraints?	Waiting time (WT)	Age at first reproduction	IO *vs*. PMO	WT decreases
	Added weight (ΔW)	Body weight at first reproduction		ΔW decreases
2 – Is ID purged under mating constraints?	Juvenile ID	Juvenile survival	IO *vs*. PMO and MAO	ID decreases
	Adult ID			
	Growth	Body weight at 45 days	MAO *vs*. MAS	ID decreases
	Female reproduction	Proportion egg-laying females	MAO *vs*. MAS	ID decreases

		Female fecundity		
		Survival of maternal offspring		
	Female copulation behaviour	Probability of being mounted	MAO *vs*. MAS	ID decreases
		Time in female position		
		N copulations as female		
	Male reproduction	Probability to sire offspring	MAO *vs*. MAS	No difference in ID
		Mate offspring production		
		Paternity share		
	Male copulation behaviour	Probability of mounting	MAO *vs*. MAS	No difference in ID
		Time in male position		
		N copulations as male		
3 – Does male allocation decreases under mating constraint?	Female reproduction	Proportion of egg-laying females	MAO *vs*. MAS	Trait value increases
		Female fecundity		
		Survival of maternal offspring		
	Female copulation behaviour	Probability of being mounted	MAO *vs*. MAS	Trait value decreases

	Time in female position		Trait value decreases
	N copulations as female		Trait value decreases
Male reproduction	Probability to sire offspring	MAO *vs*. MAS	Trait value decreases
	Mate offspring production		Trait value decreases
	Paternity share		Trait value decreases
Male copulation behaviour	Probability of mounting	MAO *vs*. MAS	Trait value decreases
	Time in male position		Trait value decreases
	N copulations as male		Trait value decreases

Table 2. Results of linear models on all traits. All models used Gaussian error term unless otherwise stated in the first column. For the line type, treatment and their interaction (fixed) effects, we report the result of likelihood-ratio tests (Chi-square statistics and associated *P*-values; one degree of freedom unless otherwise stated). When the interaction was significant we tested for line type effects separately within each treatment, and for treatment effects separately within each line type. Differences between treatment means (after correction for block effects) are provided in the "Estimates" column (WT: waiting time; ΔW: added growth; ID: inbreeding depression). ID was estimated in the original scale, irrespective of the link functions or transformations used to normalize the data in statistical models. Additional terms were considered in the models, including replicate within line type, family within replicate and their interactions with treatments (all random), block (fixed effect) and covariates describing the albino partners for male reproductive traits. Starting from a full model, we removed non-significant additional terms – the remaining significant terms (included in the final model) are listed in the last column (Chi-square, degree of freedom and associated *P*-values). When the effect of focal body weight was significant, models were run with (results in italics) and without (no italics) body weight as a covariate. Codes are F (family), R (replicate), T (treatment), B (block), W (focal body weight), MW (body weight of female mate), MO (mate origin, line $C1_{alb}$ or $C2_{alb}$) and CW (body weight of male competitor).

Trait	Treatments compared	Line type	Treatment	Estimates	Treatment x line type	Additional terms
Traits indicating the resistance to self-fertilization						
Age at first reprod. (days)	IO / PMO	IO: 10.57 (**0.001**) PMO: 0.015 (0.90)	S: 6.81 (**0.009**) C: 34.17 (**<0.001**)	WT(S) = 6.54 +/- 2.2 WT(C) = 16.5 +/- 2.2	11.49 (**<0.001**)	F*T (7.36;2;0.014) B (11.01;2;0.004)
Body weight at first reprod. (mg)	IO / PMO	IO: 11.51 (**<0.001**) PMO: 1.36 (0.24)	S: 12.23 (**<0.001**) C: 25.38 (**<0.001**)	ΔW(S) = 18.5 +/- 4.8 ΔW(C) = 51.7 +/- 5.9	11.7 (**<0.001**)	F*T (7.47;2;0.015) B (9.97;2;0.007)
Juvenile fitness and inbreeding depression						
Juvenile survival (1st experiment) - Binomial	IO / PMO / MAO	IO: 8.77 **(0.003)** PMO: 2.88 (0.09) MAO: 0.07 (0.79)	S: 4.91 (2df, 0.09) C: 28.8 (2df, <**0.001)**	ID(S) = 0.03 +/- 0.10 ID(C) = 0.28 +/- 0.10	7.38 (2df;0.025)	B (9.78;2;0.008)
Juvenile survival (2nd experiment) - Binomial	Selfed / outcrossed	Self: 5.54 **(0.019)** Out: 16.5 **(<0.001)**	S: 3.37 (0.07) C: 47.2 **(<0.001)**	ID(S) = 0.11 +/- 0.12 ID(C) = 0.50 +/- 0.11	18.1(<0.001)	None

Adult reproduction and inbreeding depression

Body weight at 45 days (Log(mg))	MAO / MAS	5.70 **(0.017)**	5.12 **(0.023)**	ID = 0.135 +/- 0.06	0.03 (0.86)	F (6.33;1; 0.006) B (37.3;5;<0.001)
Proportion of egg-laying females (Binomial)	MAO / MAS	0.01 (0.92) *2.71 (0.10)*	0.78 (0.38) *0.03 (0.87)*	ID = 0.054 +/- 0.06 *ID = 0.009 +/- 0.045*	0.08 (0.78) *0.75 (0.39)*	None *W (67.4;1;<0.001)*
Female fecundity (Log(eggs/day))	MAO / MAS	1.31 (0.25) *0.30 (0.58)*	8.06 **(0.004)** *5.86 **(0.015)***	ID = 0.174 +/- 0.06 *ID = 0.135 +/- 0.05*	0.80 (0.37) *0.09 (0.76)*	R (12.5;1; <0.001) B (12.6;5; 0.027) *R (9.22;1;0.003)* *B (27.3;5;<0.001)* *W (48.3;1;<0.001)*
Survival of maternal offspring (Binomial)	MAO / MAS	1.76 (0.19) *0.01 (0.90)*	3.21 (0.07) *1.59 (0.20)*	ID = 0.11 +/- 0.06 *ID = 0.08 +/- 0.06*	0.19 (0.67) *0.37 (0.54)*	B (21.3;5;<0.001) *F*T (6.83;2;0.03)* *W (26.0;1;0.001)*
Female reproduction (Log(offspr/day))	MAO / MAS	1.66 (0.20)	6.42 **(0.011)**	ID = 0.20 +/- 0.07	0.20 (0.65)	R (5.73;1;0.006) B (25.7;5;<0.001)

		0.43 (0.50)	*5.20* ***(0.023)***	*ID = 0.13 +/- 0.07*	*0.02 (0.89)*	*R (3.31;1;0.034)* *B (24.8;5;<0.001)* *W (28.2;1;<0.001)*
Probability to sire offspring (per female mate) (Binomial)	MAO / MAS	0.09 (0.76)	17.93 **(< 0.001)**	ID = 0.23 +/- 0.05	1.32 (0.25)	B (11.6;5; 0.04) CW (8.0;1; 0.004) MO (6.5;1; 0.011)
		0.53 (0.47)	*13.2* ***(< 0.001)***	*ID = 0.20 +/- 0.05*	*1.37 (0.24)*	*W (18.2;1; <0.001)* *CW (4.1;1; 0.04)* *MO (8.8;1; 0.003)*
Mate offspring production (offspr/day)	MAO / MAS	2.32 (0.12)	2.21 (0.14)	ID = 0.072 +/- 0.051	0.47 (0.49)	B (18.9;5; 0.002) MW (17.2;1; 0.001) MO (7.48; 1;0.006)
Paternity share (Log, when > 0)	MAO / MAS	MAS: 8.81 **(0.002)** MAO: 0.43 (0.51)	S: 28.6 (**< 0.001**) C: 3.78 (0.052)	ID(S) = 0.56 +/- 0.07 ID(C) = 0.21 +/- 0.10	8.28 **(0.004)**	CW (8.62;1;0.003) MO (4.87;1; 0.027)
		MAS: 6.44	*S: 28.2 (***< 0.001***)* *C: 3.61 (0.057)*	*ID(S) = 0.53 +/- 0.08* *ID (C) = 0.20 +/- 0.11*	*8.41* ***(0.004)***	*W (5.92;1; 0.015)* *MO (4.35;1;0.037)*

		(0.011)				*CW (9.18;1; 0.002)*
		MAO: 2.68				
		(0.102)				
Male reproduction (Log(1+offspr/day / mate))	MAO / MAS	MAS: 8.08 (**0.004**)	S: 36.3 (**< 0.001**)	ID(S) = 0.706 +/- 0.059	4.09 (**0.043**)	B (11.42;5;0.043)
		MAO: 1.28 (0.25)	C: 6.93 (**0.008**)	ID(C) = 0.485 +/- 0.103		CW (23.5;1; <0.001)
		0.00 (0.97)	*36.0 (**< 0.001**)*	*ID = 0.59 +/- 0.08*	*3.65 (0.056)*	*W (17.8;1; <0.001)*
						MW (9.53;1;0.002)
						CW (12.2;1; <0.001)
<u>Copulation behavior</u>						
Probability to be mounted (Binomial)	MAO / MAS	0.14 (0.70)	0.00 (0.99)	ID = 0.085 +/- 0.07	2.19 (0.14)	F*T (6.5;1; 0.039)
Time in female position (s, when	MAO / MAS	0.22 (0.63)	0.01 (0.92)	ID = 0.014 +/- 0.03	0.04 (0.85)	F (10.0;1;0.001)
						B (11.1;5;0.048)

> 0)		*0.006 (0.94)*	*0.02 (0.87*)	*ID = 0.065 +/- 0.04*	*0.00 (0.99)*	*F (6.9;1;0.004)* *B (14.8;5; 0.011)* *W (14.6;1; <0.001)*
N female copulations (Poisson)	MAO / MAS	0.48 (0.49)	0.48 (0.49)	ID= -0.13 +/- 0.15	0.54 (0.46)	None
Probability of mounting (Binomial)	MAO / MAS	0.14 (0.70) *1.55 (0.21)*	3.36 (0.07) *1.96 (0.16)*	ID = 0.17 +/- 0.04 *ID = 0.12 +/- 0.08*	0.84 (0.36) *0.51 (0.47)*	None *W (23.2 ;; 0.001)*
Time in male position (s, when > 0)	MAO / MAS	0.36 (0.55) *1.53 (0.22)*	0.34 (0.56) *0.19 (0.66)*	ID = -0.09 +/- 0.125 *ID = -0.08 +/- 0.13*	0.20 (0.66) *0.29 (0.59)*	F (3.33;1 ; 0.034) *W (1.09;1;0.043)* *F (3.33;1 ; 0.034)*
N male copulations (Poisson)	MAO / MAS	0.61 (0.44) 0.03 (0.85)	0.60 (0.44) *0.29 (0.59)*	ID = 0.15 +/- 0.14 ID = 0.10 +/- 0.14	0.50 (0.48) *0.56 (0.45)*	None *W (5.96;1; 0.015)*

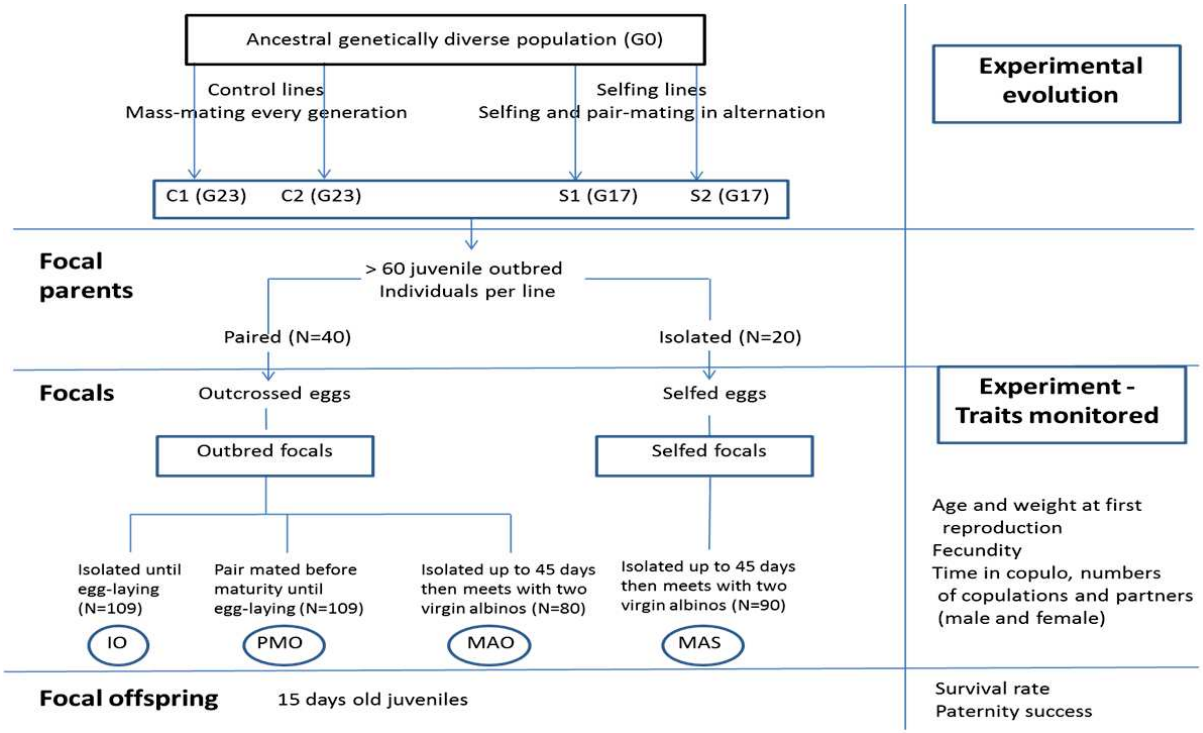
Ancestral genetically diverse population (G0)
Control lines
Mass-mating every generation
Selfing lines
Selfing and pair-mating in alternation
Experimental evolution
C1 (G23)
C2 (G23)
S1 (G17)
S2 (G17)
Focal parents
> 60 juvenile outbred Individuals per line
Paired (N=40)
Isolated (N=20)
Focals
Outcrossed eggs
Selfed eggs
Experiment - Traits monitored
Outbred focals
Selfed focals
Isolated until egg-laying (N=109)
Pair mated before maturity until egg-laying (N=109)
Isolated up to 45 days then meets with two virgin albinos (N=80)
Isolated up to 45 days then meets with two virgin albinos (N=90)
Age and weight at first reproduction
Fecundity
Time in copulo, numbers of copulations and partners (male and female)
IO
PMO
MAO
MAS
Focal offspring
15 days old juveniles
Survival rate
Paternity success

Figure 1

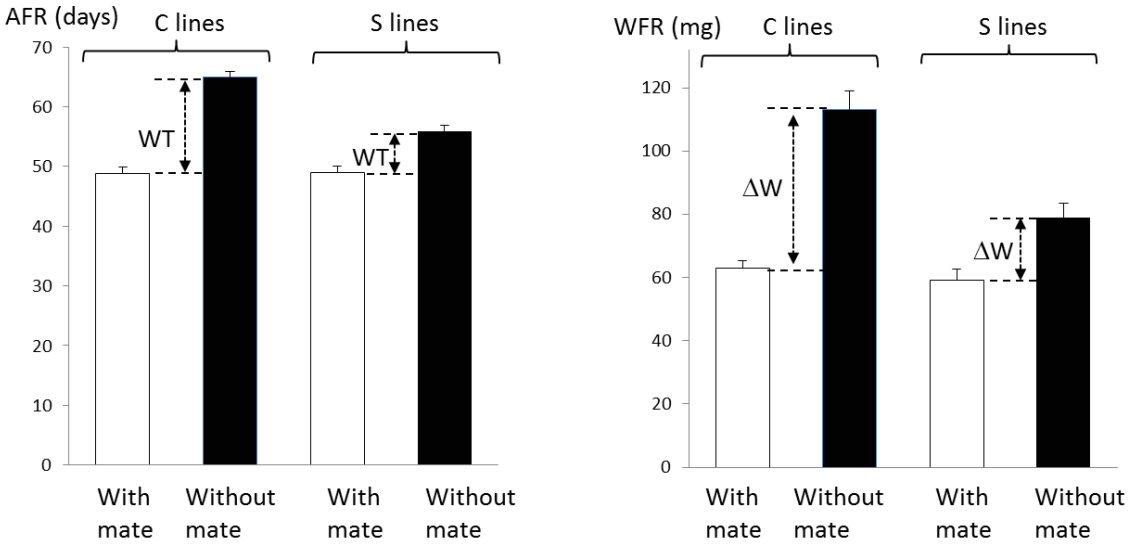
AFR (days)
C lines
S lines
70
60
50
40
30
20
10
0
WT
WT
With mate
Without mate
With mate
Without mate
WFR (mg)
C lines
S lines
120
100
80
60
40
20
0
ΔW
ΔW
With mate
Without mate
With mate
Without mate

Figure 2

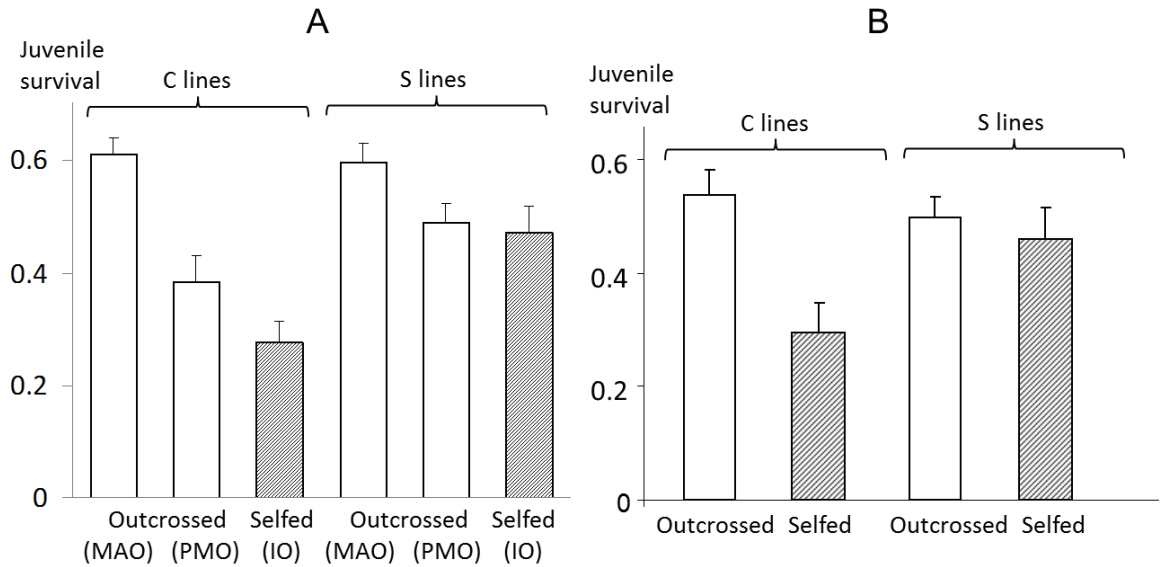
A
Juvenile survival
C lines
S lines
0.6
0.4
0.2
0
Outcrossed (MAO)
Outcrossed (PMO)
Selfed (IO)
Outcrossed (MAO)
Outcrossed (PMO)
Selfed (IO)
B
Juvenile survival
C lines
S lines
0.6
0.4
0.2
0
Outcrossed
Selfed
Outcrossed
Selfed

Figure 3

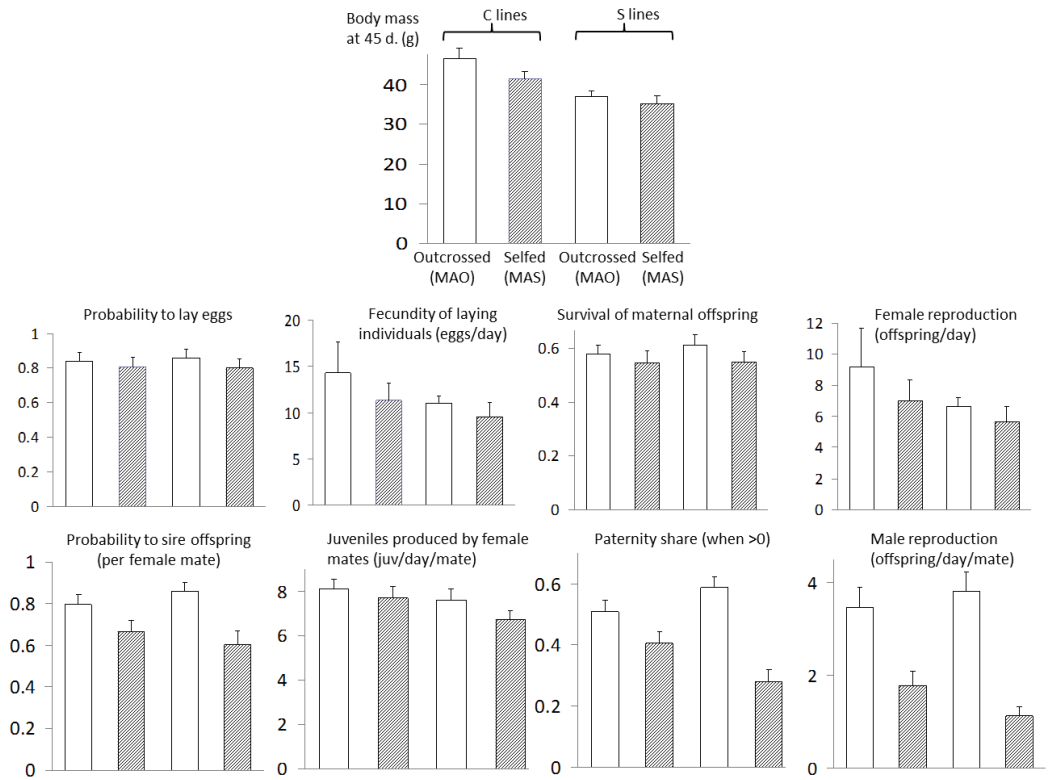
Body mass at 45 d. (g)
C lines
S lines
40
30
20
10
0
Outcrossed (MAO)
Selfed (MAS)
Outcrossed (MAO)
Selfed (MAS)
Probability to lay eggs
Fecundity of laying individuals (eggs/day)
Survival of maternal offspring
Female reproduction (offspring/day)
Probability to sire offspring (per female mate)
Juveniles produced by female mates (juv/day/mate)
Paternity share (when >0)
Male reproduction (offspring/day/mate)

Figure 4

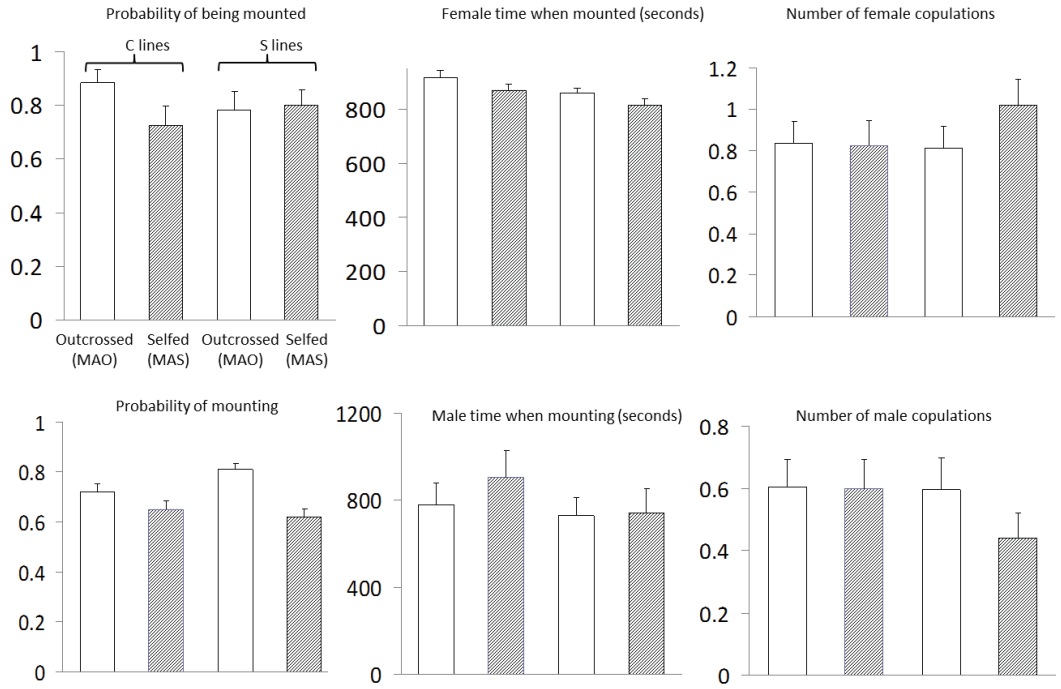
Probability of being mounted
C lines
S lines
1
0.8
0.6
0.4
0.2
0
Outcrossed (MAO)
Selfed (MAS)
Outcrossed (MAO)
Selfed (MAS)
Female time when mounted (seconds)
800
600
400
200
0
Number of female copulations
1.2
1
0.8
0.6
0.4
0.2
0
Probability of mounting
1
0.8
0.6
0.4
0.2
0
Male time when mounting (seconds)
1200
800
400
0
Number of male copulations
0.8
0.6
0.4
0.2
0

Figure 5

Supporting information – Noel et al. – Reduced mate availability leads to evolution of self-fertilization and purging of inbreeding depression in a hermaphrodite

Figure S1. Scenario for an evolutionary transition from outcrossing to selfing. It starts with an outcrossing population with large inbreeding depression (ID), strong resistance to selfing (e.g., long waiting time) and equal male / female sex allocation, which is at some point constrained to self-fertilize (e.g., because mates or pollinators are lacking). If this regime is maintained for a long-enough time, ID is purged, and both the resistance to selfing and male / female sex allocation decrease. The selfing syndrome (see Text) can also evolve. When ID falls below a threshold (e.g., ½ in classic genetical models; dotted line), the population can evolve towards preferential (unconditional) selfing, and maintains this state even if mating constraints are suppressed.

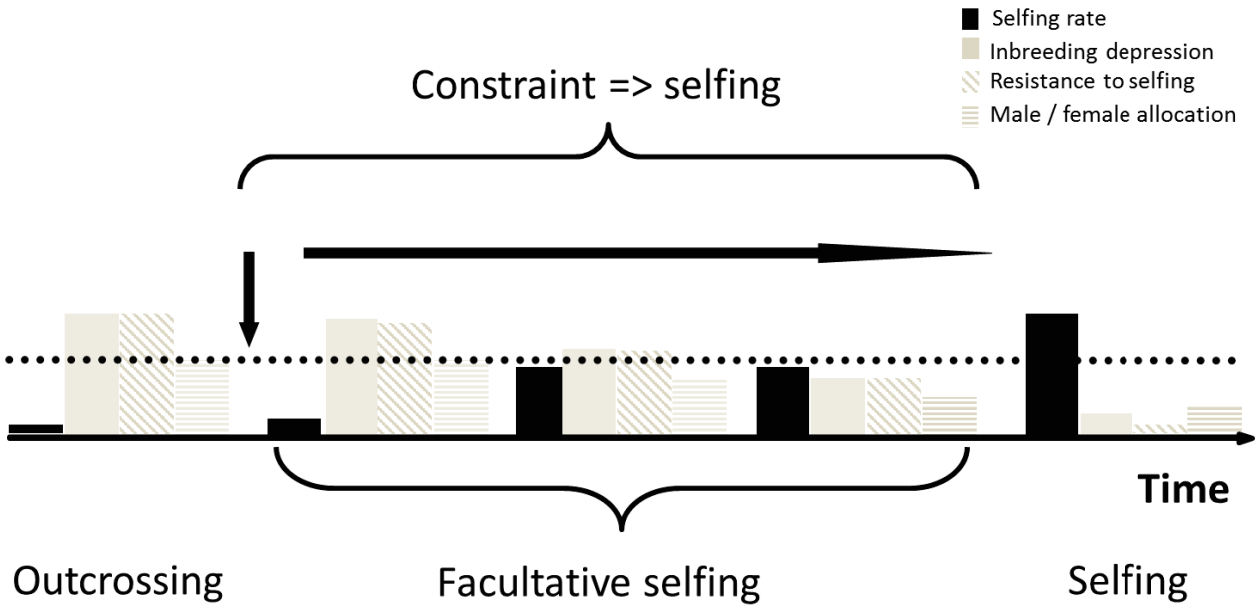

Table S1. Some characteristics of the sites (all located near Montpellier, southern France) in which *Physa acuta* individuals were sampled to constitute the base population from which the experimental lines were derived. Environments are either open (O; rivers) or closed (C; ponds), and can be permanent (P) or temporary (T) in terms of water availability (see Bousset et al. 2004[1]).

[1] Bousset, L., P.-Y. Henry, P. Sourrouille, and P. Jarne. 2004. Population biology of the invasive freshwater snail *Physa acuta* approached through genetic markers, ecological characterization and demography. Mol. Ecol. 13: 2023-2036.

Site	City	GPS coordinates	Environment
Lamalou	Saint-Martin-de-Londres	43°47’N 3°43’E	O P
Salaison	Teyran	43°41’N 3°55’E	O P
Yorgues	Saint-Jean de Cuculles	43°44’N 3°50’E	O T
Mosson	Vailhauquès	43°39’N 3°45’E	O T
Cambou	Viols-en-Laval	43°45’N 3°43’E	C P
Seuilles	Cazevieille	43°45’N 3°46’E	C P
Cazevieille	Cazevieille	43°46’N 3°47’E	C P
Viols-le-fort	Viols-le-Fort	43°44’N 3°42’E	C T
Roubiac	Cazevieille	43°45°N 3°47’E	C T
Cazevieille	Cazevieille	43°46’N 3°48’E	C T
Fertalière	Cournonterral	43°34’N 3°40’E	C T
Jasses	Notre-Dame-de-Londres	43°51’N 3°46’E	C T
Bouillés	Cournonterral	43°34’N 3°41’E	C T
Gourg de la Lergue	Lodève	43°44’N 3°19’E	O P

Table S2. Mean values of the traits studied per line (S and C) and treatment (IO, PMO, MAO and MAS). N is the number of data points (observations) and s.d. the standard deviation. Means are corrected for both block effects and covariates that account for variation in the origin and quality of potential mates, when they are significant.

	Trait	**Line**	**Treatment**	**N**	**Mean**	**s.d.**
Traits indicating the resistance to self-fertilization	Age at first reproduction (days)	S	IO	53	55.9	13.06
			PMO	52	49.0	7.60
		C	IO	56	64.9	13.40
			PMO	57	48.9	6.35
	Body weight at first reproduction (mg)	S	IO	46	78.8	31.9
			PMO	46	59.1	24.05
		C	IO	48	113.3	40.0
			PMO	47	63.3	15.94
Juvenile fitness and inbreeding depression	Juvenile survival (15 days)	S	IO	44	0.471	0.309
	First experiment		PMO	48	0.487	0.255
			MAS	44	0.514	0.302
			MAO	42	0.595	0.232
		C	IO	40	0.276	0.241
			PMO	48	0.384	0.321

			MAS	42	0.559	0.295
			MAO	42	0.611	0.194
	Juvenile survival (15 days)	S	selfed	39	0.449	0.175
	Second experiment		outcrossed	85	0.503	0.188
		C	selfed	36	0.309	0.211
			outcrossed	86	0.622	0.189
Adult reproduction and inbreeding depression	Body weight at 45 days (mg)	S	MAS	56	35.22	14.50
			MAO	49	36.96	10.34
		C	MAS	53	41.40	15.16
			MAO	52	46.67	18.80
	Proportion of egg-laying females	S	MAS	55	0.80	0.40
			MAO	50	0.86	0.35
		C	MAS	52	0.81	0.39
			MAO	50	0.84	0.37
	Female fecundity	S	MAS	42	9.60	4.42
	(N eggs per day; zero excluded)		MAO	41	11.05	5.11
		C	MAS	52	11.34	5.93
			MAO	50	14.30	8.04
	Female reproduction	S	MAS	39	6.33	3.57
	(offspring per day; zero excluded)		MAO	42	6.66	3.49
		C	MAS	40	6.98	4.65

			MAO	41	9.18	5.86
	Probability to sire offspring	S	MAS	106	0.60	0.51
	(per female mate)		MAO	90	0.86	0.40
		C	MAS	98	0.67	0.53
			MAO	96	0.80	0.46
	Mate offspring production	S	MAS	106	7.28	4.19
	(per day; zero excluded)		MAO	90	8.24	4.19
		C	MAS	98	8.23	4.61
			MAO	96	8.44	4.00
	Paternity share	S	MAS	63	0.25	0.28
	(per mate; zero excluded)		MAO	77	0.56	0.31
		C	MAS	67	0.43	0.31
			MAO	76	0.54	0.32
	Male reproduction	S	MAS	112	3.47	1.87
	(offspring per day and mate)		MAO	97	3.81	3.48
		C	MAS	103	1.79	2.40
			MAO	104	3.47	3.55
Sex allocation	Probability to be mounted	S	MAS	50	0.80	0.16
			MAO	37	0.78	0.17
		C	MAS	40	0.725	0.20
			MAO	43	0.88	0.10

Time in female position	S	MAS	40	812.2	190.3
(s / 45mn ; zero excluded)		MAO	29	861.5	109.8
	C	MAS	29	868.6	219.8
		MAO	38	918.1	297.8
N copulations as female	S	MAS	50	1.02	0.89
		MAO	37	0.81	0.66
	C	MAS	40	0.825	0.78
		MAO	43	0.84	0.69
Probability of mounting	S	MAS	50	0.62	0.24
		MAO	37	0.81	0.15
	C	MAS	40	0.65	0.23
		MAO	43	0.72	0.20
Time in male position	S	MAS	31	782.0	460.5
(s / 45mn ; zero excluded)		MAO	30	906.8	606.2
	C	MAS	26	728.3	416.2
		MAO	31	740.5	577.7
N copulations as male	S	MAS	50	0.44	0.58
		MAO	37	0.59	0.64
	C	MAS	40	0.60	0.59
		MAO	43	0.60	0.58

Chapitre III Régime de reproduction et Potentiel Adaptatif.

Après avoir observé qu'une possibilité de début de transition pouvait être atteinte dans nos lignées S d'évolution expérimentale, nous avons voulu tester si le potentiel adaptatif était à ce stade réellement différent, et si en forçant l'autofécondation cette fois ci sans alternance, nos lignées S aurait un avantage sur les lignées C, en raison de leur évolution préalable (purge partielle de la dépression de consanguinité et diminution du temps d'attente, donc de la « résistance » à l'autofécondation). Nous avons pour cela choisi de sélectionner artificiellement un trait morphologique : le ratio l/ L de la coquille (voir Chapitre IA) dans nos deux lignées et dans deux traitements de reproduction différents : allofécondation stricte (Out) et autofécondation stricte (Self). Cette expérience d'une durée de 21 mois a été réalisée entre novembre 2013 et juillet 2015 et s'étale sur 7 générations, dont 6 générations de sélection. Chaque génération, j'ai mesuré la réponse à la sélection pour chacun des 4 groupes formés (C/Out, C/Self, S/Out, S/Self) ce qui nous permet d'estimer la différence entre les groupes et l'évolution de cette différence dans le temps. Les résultats et conclusions de cette expérience sont énoncés dans la partie ci-dessous.

Classification: Biological sciences - Evolution

Experimental evidence for the negative effects of self-fertilization on the adaptive potential of populations

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Author contributions: EN, PJ, SG and PD designed the study, interpreted the results and wrote the paper. EN, AM, AS, VS and PD performed the experiments and genetic analyses. EN and PD monitored the experimental evolution lines and analyzed the results.

Significance

Self-fertilization is believed to be an evolutionary dead-end, impairing adaptation and increasing the risk of population extinction, and this idea is sustained by theoretical and phylogenetic analyses. Here we directly measure how selfing affects the response of a quantitative trait to selection in a snail species over the course of generations. We show that outcrossing populations respond better to selection than selfing ones over a few generations, irrespective of the occurrence of recurrent selfing in their past history (30 generations), providing some experimental asset to the dead-end hypothesis. Our study might serve as a reference for future studies on the influence of recurrent selfing on the adaptive potential.

Abstract

Self-fertilization is widely believed to be an evolutionary dead-end, impairing adaptation and increasing the risk of extinction. However we lack direct measurements of how selfing affects the response to selection within species over the course of generations. Selfing may initially increase the available genetic variance by putting alleles in the homozygous state, but can on a longer term decrease it, as it enhances genetic drift and makes recombination inefficient. We study the response to selection on a morphological trait in laboratory populations of a self-compatible hermaphroditic snail under either selfing or outcrossing. Both treatments are applied to two types of populations: some having undergone frequent selfing and purged a substantial fraction of their mutation load in their recent history, others continuously maintained under outcrossing. As expected, populations with a history of outcrossing respond faster to selection than those which have experienced selfing before selection started. In addition, when self-fertilization occurs during selection, the response is initially faster, but then rapidly slows down while outcrossing populations maintain their response throughout the experiment. This occurs irrespective of past selfing history, suggesting that having previously purged inbreeding depression does not significantly alter the effect of current self-fertilization on the response to selection. These findings confirm the short-term positive and long-term negative effects of selfing on the adaptive potential that have never been experimentally tested in animals.

Key-words: self-fertilization, adaptation, artificial selection, experimental evolution, freshwater snail, *Physa acuta*

The adaptive potential of populations, *i.e.* their ability to respond to natural selection in a changing environment, is a key issue in evolutionary biology, and is based on the additive genetic variation among individuals (Fisher 1930, Bell & Gonzalez 2009). What can alter this adaptive potential, and how it can be altered, matters for a large range of questions such as the evolution of sexual selection and of mating systems (Agrawal & Whitlock 2012, Lande & Porcher 2015) or local adaptation (Kawecki & Ebert 2004), including applied issues such as the potential to invade new ecosystems (Facon et al. 2006) or the rescue of endangered species in the face of global change (Gomulkiewicz & Holt 1995, Bell 2013).

Self-fertilization in hermaphroditic organisms displays several short-term advantages, including an increased efficiency of gene transmission across generations (Fisher 1941) and the possibility of reproducing when pollinators or partners are scarce (reproductive assurance; Baker 1955). However it is also expected to decrease the adaptive potential. Self-fertilization leads to homozygosity, with several consequences. For each locus, homozygosity decreases effective population size by up to a factor two (Pollak 1987, Caballero & Hill 1992). At the scale of the whole genome, it makes recombination inefficient, so that selfing populations are more susceptible to selective sweeps and background selection, which further reduce the effective population size (reviewed in Charlesworth and Wright 2001, Glémin and Galtier 2012, Hardfield and Glémin, submitted). The combination of higher homozygosity and weaker recombination is expected to result in a lower quantitative genetic variance in selfing than in outcrossing populations (Wright 1969, Charlesworth & Charlesworth 1995, Lande & Porcher 2015). Moreover, selfing populations show a decreased ability to fix several advantageous alleles at the same time, a characteristic that they share with asexual populations (Muller 1932, Weissman & Barton 2012). On the whole, the capacity to respond to environmental changes is expected to be depressed by selfing (Glémin & Ronfort 2013), and autogamous species are thought to adapt more slowly and to go extinct more often than

allogamous ones. This is consistent with the phylogenetic distribution of selfing plants, mainly observed on terminal branches of phylogenies, which strongly suggest that they have higher extinction rates than do outcrossers (Goldberg et al. 2010, Igic & Busch 2013). Phylogenetic and population genomic analyses also support a lower efficacy of selection in selfing lineages of plants (Hazzouri et al. 2013), animals (Burgarella et al. 2015) and fungi (Gioti et al. 2013) backing the old idea that selfing constitutes an evolutionary dead-end on the long term (Stebbins 1957, Igic & Busch 2013, Lande & Porcher 2015). However these lines of evidence are correlative and biased towards species that are not (yet) extinct, and direct experimental study of how selfing affects the adaptive potential compared to outcrossing, all else being equal, is still scarce. Morran et al. (2009) observed that isogenic lines of *Caenorhabditis elegans* under artificially elevated mutation and/or changes in environment, tended to adapt less well under obligate selfing than under partial outcrossing. However, isogenic lines do not mimick natural populations because they have no standing variation to start with, and only rely on new mutations. In these conditions, the short-term benefit of selfing on quantitative genetic variance is reduced.

To test the hypothesis that selfing reduces the adaptive potential, we developed an experimental evolution approach, which has previously been used successfully in a few studies to evaluate some aspects of the evolution of self-fertilization in both plants (Bodbyl Roels & Kelly 2011) and animals (Morran et al. 2009, Chelo et al. 2014). It was conducted in the outcrossing freshwater snail *Physa acuta*, a species that has widely been used for studying the evolution of mating systems (Henry et al. 2005, Escobar et al. 2009, 2011). We compared experimental lines that have been constrained to self-fertilize every other generation during *ca*. 30 generations (S lines) with fully outcrossed control lines (C lines), a situation representative of an incipient transition from outcrossing to selfing (medium-term evolution).

These lines have already evolved several traits associated with the "selfing syndrome", which includes purging a significant part of their inbreeding depression (Noel et al. submitted).

Artificial selection was applied on a morphological (shell) trait over six generations (short-term evolution) – the response to selection is here considered as a proxy of the adaptive potential that relies on standing variation (*i.e.* neglecting mutational input) after medium-term evolution in S and C lines. The mating system of individuals during the selection experiment should also affect the response to selection because selfing can speed up short-term selection by exposing recessive mutations and generating more additive genetic variance (Charlesworth & Charlesworth 1995, Lande & Porcher 2015). On the other hand inbreeding depression, in the lines that have not previously purged it, should reduce the efficiency of selection under selfing. We thus applied selection under both an outcrossing (Out) and a selfing (Self) regimes in both line types to test how both the history of selfing and the current mating system affect the response to selection.

Results

Selection on shell shape was conducted over six generations using individuals from two types of experimental lines (S and C) that were either selfing, or outcrossing, leading to four treatments (C/Self, C/Out, S/Self and S/Out). This protocol allows to test several predictions: (i) The comparison of the S and C lines under an outcrossing regime (C/Out *vs*. S/Out) allows to test the hypothesis that selfing reduces the standing variation available for adaptation. (ii) Selfing should lead to a temporary increase in variance compared to outcrossing in both line types. (iii) Because they have previously purged part of their inbreeding depression, S lines should benefit from this increase more than C lines and maintain their response for a longer time under selfing.

Fifty individuals out of 200 were selected per generation, line and treatment, resulting in more than 12000 individuals measured over the whole experiment. In all conditions the

W/L (width/length) shell ratio (hereafter shell roundness) significantly increased over generations in response to selection as shown by regression analysis (Table 1 and Fig. 1A). However the phenotypic values did not increase linearly. A maximum-likelihood piecewise regression indeed detected a breakpoint (hereafter BP) at the third generation (2.81, 95% CI: 2.70 - 3.11), meaning that the rate of phenotypic change per generation changed in some populations around the third generation of selection (Table 1). This rate was affected by treatment, selfing populations initially responded faster than outcrossing ones ('treatment*generation before BP': $P = 1.2 \times 10^{-6}$), but were slower afterwards ('treatment*generation after BP' interaction: $P = 0.018$). Outcrossing populations indeed did not show any marked breakpoint (Fig. 1A). The rate of phenotypic change per generation was also on average higher in the C than in the S lines both before and after the BP ('line type*generation': $P = 0.015$ and < 0.001 respectively). We did not detect any significant interaction between line type and treatment both before and after the BP (three-way interactions in Table 1) although the difference in slopes appeared visually larger in C (C/Self *vs*. C/Out) than in S (S/Self *vs*. S/Out) lines, especially before the BP (Fig. 1A).

All lines experienced the same selection intensity since a constant fraction of the shell roundness distribution (upper quartile) was selected per generation. Differences in the response to selection may therefore arise for two non-exclusive reasons: (i) the phenotypic variance may be larger in some lines resulting in a larger selection differential (*i.e.* the average difference in roundness between the upper quartile and the whole distribution); (ii) the fraction of variance that is genetically transmitted over generations may be larger in some lines (*i.e.* a higher phenotypic change per unit of selection differential). In order to disentangle these two effects we computed selection differentials and plotted both the phenotypic values against cumulative selection differentials CSD (Fig. 1B) and the CSD against generations (Fig. 1C), taking into account the previously-detected breakpoint.

The change in phenotype per unit of CSD was lower under selfing than under outcrossing at all times, and more so after the BP (P = 0.0006 and 1.5 x 10^{-5}, before and after BP respectively; Fig. 1B and Table 2). Before the BP, the two-way (Line*CSD) and three-way (Line*treatment*CSD) interactions were also significant, reflecting a stronger response in C than in S lines, this difference being more attenuated under selfing. After the BP, C and S lines showed similar responses, and were reduced by selfing in the same way. In contrast with the phenotypic response, the increase in CSD over generations seemed to be linear (Fig. 1C and Table 3), although a BP was imposed at generation 3 in the regression (estimated slopes of 0.0295 and 0.0286 before and after the breakpoint respectively). The significant treatment*generation interactions (P= 1.9 x 10^{-9} and 8.4 x 10^{-12} before and after BP respectively) indicates a stronger per-generation selection differential in the selfing than in the outcrossing treatment. An even stronger effect is detected in the S lines before the breakpoint ('line*treatment*generation before BP' interaction: P= 0.005).

The pattern of change in CSD with generations matched that of phenotypic variance (compare Fig. 1C and *SI Appendix*, Fig. S3). The latter was indeed consistently larger in selfing than in outcrossing treatments throughout the experiment (*SI Appendix*, Figs. S3 and S4, Table S2). The phenotypic variance (*SI Appendix*, Fig. S3) tended to increase before the BP in selfing populations, and then decrease. The reverse pattern was observed in the S/O line (first decrease then increase) while the phenotypic SD uniformly decreased in the C/O line (see significant 'treatment*generation' interactions in *SI Appendix*, Table S2).

To assess the effect of drift prior to and during the experiment, we estimated genetic diversity at 10 microsatellite loci at generations G0 and G6. The S lines were initially slightly less variable than C lines (0.271 vs 0.376 on average at the G0) and after the six generations of selection all lines had lost some diversity. However the selfing treatment induced a

stronger loss of diversity than the outcrossing treatment (0.206 vs 0.268 on average over all lines) (Fig S5).

Discussion

Self-fertilization has long been proposed to be an evolutionary dead-end (Baker 1955) based on theoretical arguments, but also on genetical and phylogenetic results (Stebbins 1957, Igic & Busch 2013, Lande & Porcher 2015). We however have little direct empirical evidence for such a claim deriving from experimental studies in which the evolution of selfing populations is followed and their adaptive potential estimated at both short-term (a few generations) and medium-term (a few tens of generations). When an outbred population switches to continuous self-fertilization, an increase in additive genetic variance (up to two-fold after a few generations) is expected (Wright 1969). Simultaneously some components of phenotypic variance, although they do not necessarily increase, become heritable. Dominance effects start to contribute to the response to selection as homozygosity is passed on across generations. This is also the case of epistatic effects as recombination progressively becomes inefficient at dissipating inter-locus associations (Charlesworth & Charlesworth 1995, Lande & Porcher 2015). On the whole, we expect a rapid increase in the response to selection in selfing populations. However, this increase should be temporary as both homozygosity and inefficient recombination also reduce the effective size, hastening the erosion of genetic variance by the combined effects of drift and selection. .

Our results meet these expectations: the response to selection was faster under self-fertilizing than under outcrossing over the first generations. This enhanced response was transitory and the response considerably slowed down after the third generation compared to the outcrossing situation. After six generations, outcrossing populations have already filled up the phenotypic gap accumulated during the first three generations (Fig. 1A). Selfing also

decreases the genetic variance in the absence of strong selection, as attested by the effect of the past mating history of our selected populations – they derived from the same initial stock, but were maintained either by pure outcrossing (C lines) or by alternate selfing and outcrossing (S lines) for *ca*. 30 generations. Here again we observe that the additive genetic variance is reduced in lines with a history of selfing, as their response to selection (under outcrossing) is much slower. As no selection was applied on shell roundness over the 30 generations prior to selection, this loss of additive genetic variance in S lines therefore reflects mostly how recurrent selfing modifies genetic variance for any quantitative phenotype. S lines have a mean realized heritability a quarter lower than C lines (0.23 vs 0.3). This is consistent with the lower effective population size of partially selfing populations, and their sensitivity to background selection and selective sweeps (Charlesworth and Wright 2001, Glémin and Galtier 2012). We indeed observed a stronger loss of neutral molecular variation in our experimental lines under selfing than under outcrossing (Fig. S5). More generally selfing populations or species are usually less genetically variable than outcrossing ones at both neutral markers (Hamrick & Godt 1991, Jarne 1995, Nyborn, 2004, Glémin et al. 2006) and phenotypic traits (Charlesworth & Charlesworth 1995).

Our protocol also allows to evaluate the interference between artificial selection on a particular trait and natural selection on deleterious mutations during the response to selection. The S lines have purged a large part of their inbreeding depression because of repeated inbreeding in their history (Noël et al. submitted). They should therefore enjoy an advantage over C lines in the selfing treatment (C/Self) when selecting on shell roundness. The C/Self lines indeed had to respond to two selective pressures at the same time: the artificial selection on shell roundness and the elimination of recessive mutations, suddenly made homozygous and exposed to selection. The interference between these two processes was expected to hamper the response to selection in the C/Self lines when compared to the S/Self ones. Our

results provided only partial evidence for this effect. While it seems that the response is initially enhanced by selfing to a greater extent in S than in C lines, it is later more reduced, The three-way interactions (indicating the differential impact of mating treatment on the response to selection per unit of CSD in C vs S lines) are significant during the first three generations only. In addition the loss of neutral molecular diversity under selfing is not more pronounced in C than in S lines. A possible explanation is that four generations of continuous selfing (one of them having taken place before selection started) were sufficient for C lines to purge their genetic load to a level that approaches that of S lines. At any rate, a previous experience in self-fertilization does not seem to prevent the rapid erosion of genetic variance under selfing.

Our analysis also returns an interesting result when considering the phenotypic variance during the response to selection. This phenotypic variance was indeed consistently higher in selfing than in outcrossing lines (*SI Appendix*, Fig. S3), resulting in a higher selective differential per generation (Fig. 1C). This is consistent with the predicted increase in genetic variance under selfing. However these results seem at odds with the expectation that a larger fraction of the phenotypic variance (including dominance and interaction effects) should be heritable under selfing. Indeed the response to selection of selfing lines became slower than that of outcrossing ones after three generations of selection (breakpoint), while the variance and selection differentials remained higher. Thus, selection became less efficient under selfing selection, in terms of phenotypic progress per unit of CSD; Over the six generations of selection, this efficiency is 0.3 in outcrossing lines (which in this case represents narrow-sense heritability) and 0.2 in selfing ones. In other terms the heritable fraction of the phenotypic variance was decreased, not increased, by self-fertilization. This suggests that the phenotypic variance of selfing lines includes a component that is not responsive to selection. We might here call to a classical effect of inbreeding on

developmental homeostasis: accumulated inbreeding tends to increase the non-genetic component of variance through developmental "decanalization" (Lerner 1954, Fowler & Whitlock 1999). The reduced ability to respond to selection after a few generations of selfing might therefore be due to a combination of fast erosion of genetic variance and increased developmental instability.

Our protocol and results can be set in an ecological perspective, since the change in mating systems experienced by the S and C lines can be interpreted as a modification in partner availability (or pollinators if we were studying plants) over several generations – we are here coming back to the essence of ecological arguments on the evolution of selfing (Baker 1955, Goodwillie et al. 2005, Pannell et al. 2015).

The comparison of the C/Out and S/Self lines provides insights on how differently outcrossing and selfing populations or species might react to change in selective conditions. This issue is particularly important in two contexts : one is climate change, which constitutes an adaptive challenge for many contemporary organisms; the other is biological invasions, when exotic species are introduced into new environments, which requires adaptation both by the exotic species itself and by the resident species with which it interacts. Our results suggest that adaptive challenges are less likely to be overcome by selfing than by outcrossing populations because they are more prone to erosion of genetic variance by the combined action of genetic drift and selection. This will obviously vary with parameters such as the effective size of populations and the rate of selfing. Our laboratory populations are rather small (around 100 individuals before selection and 200 during selection) and the selfing rates vary between 50% (before selection started) and 100% (during selection). However, although many selfing species in nature probably have nominal population sizes higher than a few hundreds, their level of microsatellite genetic diversity is usually low, at best of the same order as that of our laboratory populations. This indicates that we did not place ourselves in

extreme low-effective size conditions. In addition the time scale at which self-fertilization limits the adaptive potential in our experiments is remarkably short. These limits are already visible after 6 generations of very high selfing rate (100%) or a few tens of generations of periodic selfing (50%). It is therefore likely that recurrent selfing reduces the adaptive potential of populations in a large range of naturally occuring selfing rates and effective population sizes. This is consistent with the observation that quantitative genetic variance tends to be lower in selfers than in outcrossers (Charlesworth & Charlesworth 1995). Together with theoretical approaches (Lande & Porcher 2015) and phylogenetic analyses (Goldberg 2010, Igic & Busch 2013), this lends credence to the idea that prolonged, predominant selfing is an evolutionary dead-end.

C/Self lines mimic an allogamous population constrained to self-fertilize and to adapt to a new environment at the same time. This situation is not unlikely in natural contexts. For example, many plant populations are facing climatic changes and loss of pollinators and/or mates (low population density) at the same time. Invasive species may experience similar challenges during colonization of new environments. In such contexts, our results suggest that although selfing provides reproductive assurance, it will, if prolonged over a few generations, increase the risk of losing genetic variation and failing to adapt. However self-fertilization increases genetic variance on the short term, temporarily boosting the response to selection. We therefore expect that plastic mating systems, able to self-fertilize during short periods in response to conditions, would have an advantage in critical situations combining environmental changes and constraints on mate or pollinator availability (Peterson & Kay 2015). This advantage must be balanced with inbreeding depression, which imposes an immediate demographic cost on selfing in populations not previously used to this mode of reproduction (*i.e.* not purged). However, populations in situations of colonization or otherwise

low density (bottlenecks), may often pay the price of inbreeding depression even under outcrossing, as mating may occur among relatives anyway.. This situation might produce a milder form of purging than selfing, but examples exist of outcrossing species that have partially purged their load during invasion or recolonization events (Pujol et al. 2009, Facon et al. 2012). So, depending on conditions, selfing might not always be the most appropriate immediate strategy in case of abrupt environmental change leading to partner / pollinator failure, but outcrossing may prevail for some time (Thomann et al. 2015). This still has to be evaluated with regard to the extinction probability of populations (Lynch & Lande 1993) and the possibility of evolutionary rescue (Gomulkiewicz & Holt 1995).

Material and methods

Overview. To examine the effect of mating systems on the response to selection we conducted an experiment in *Physa acuta,* a preferentially outcrossing freshwater snail. We compared two types of experimental evolution lines: S (frequent selfing) lines in which selfing was enforced every other generation by keeping individuals isolated and C (control) lines freely mating in large groups at each generation. Each type was represented by two replicate lines. After around 30 generations these lines were used in a selection experiment. Adults were selected for a larger shell width-to-shell length (W/L) ratio during six generations under either of two mating treatments (selfing or outcrossing). At each generation, the W/L ratio was measured in 200 juveniles per line, and the 50 individuals with the largest ratio were retained as parents for the next generation. We estimated both the response to selection and the selection differential per generation.

Lines Maintenance. The creation of the experimental evolution lines of *P. acuta* used here has already been fully described (Noël et al. submitted). Briefly, individuals were collected in

10 sites near Montpellier in 2007 from which a highly diverse ancestral population was derived through two generations of intermixing. Two types of experimental evolution lines were then derived at population size of 80 adults per generation. Each type included two replicates. Individuals from the C lines were mass-mated which results in near 100% outcrossing in this species this is confirmed molecularly y the absence of heterozygote deficiencies in the microsatellite data, fig. S5). Individuals from the S lines were constrained to self-fertilize every other generation, followed by a generation of pair-mating with a single partner. This regime was maintained for *ca*. 30 generations. The strong inbreeding depression for juvenile survival observed in natural populations was largely purged in the S lines (0.1 vs 0.5 in C lines), (Noël et al. submitted), while the inbreeding depression on adult fecundity remained (0.2). In order to start the selection experiment under two mating regimes, we extracted 200 outcrossed adult virgin snails from each line (this occurred at generations 29, 31, 35 and 34 of experimental evolution for lines S1, S2, C1 and C2 respectively) and split them into two groups, 100 for the outcrossing and 100 for the selfing treatment. In the outcrossing treatment, the 100 individuals were mass-mated and the offspring they produced were collected to enter the selection protocol as the "generation zero" (G0 of selection). In the selfing treatment, the 100 individuals underwent a generation of self-fertilization without selection to generate the G0 of the selection protocol.

Response to Selection Assay. The response to selection was evaluated on the ratio of width/length (W/L) of individual shells. This easily recordable ratio is likely to be selected under some environmental conditions; predation by crayfish may for example favor more elongated shells (Auld & Relyea 2008). However it is unlikely to be inadvertently selected under our laboratory conditions, neither to be closely associated to fitness. Its heritability was evaluated in a preliminary experiment using a full-sib design (see *SI Appendix*). Its value (h^2 =

0.17) suggested that we could expect a detectable response to selection over a few generations. We opted for selection by truncation in order to get rapid responses and to detect potential differences between lines and treatments. Selection was performed over six successive generations (21 months) on four combinations: C lines maintained under a selfing treatment (C/Self), C lines and outcrossing (C/Out), S lines and selfing (S/Self), and S lines and outcrossing (S/Out). At each generation, individuals were kept isolated and virgin until they were measured and the 25% of individuals exhibiting the largest W/L ratio were retained as parents for the next generation. Selected individuals either remained isolated until they reproduced or were mass-mated depending on treatment. The number of reproductive parents was 50 drawn out of 200 individuals. Up to 300 individuals were sometimes measured in the selfing treatment to compensate for mortality due to inbreeding depression.

Data Analysis. The response to selection was evaluated using a linear mixed model in which the individual phenotypic value was modelled as a function of the covariate "number of generations of selection", and categorical factors "experimental evolution line type" (S versus C) and "experimental treatment" (selfing versus outcrossing). Generation, line type and treatment were modelled as fixed effects including all two- and three-way interactions. We also included line replicate and replicate-by-generation interaction (when significant) as additional random effects, as well as temporal block random effect corresponding to 1-month periods starting from the beginning of the selection. As we were interested in temporal changes in the response to selection over the course of generations, we used a piecewise regression (McZgee and Carleton 1970) that allows for a single change in the slope value at some point (breakpoint) along the abscissa axis – the best breakpoint (BP) being estimated by maximum likelihood. While a piecewise-linear function is only an approximation of how selection response might change over generations, we preferred it over a quadratic function or

non-linear regressions for several reasons: first linear slopes have a more straightforward biological interpretation, especially of interaction terms, than quadratic coefficients. Second, by remaining within the framework of linear mixed models, we could incorporate the random terms associated with replicates and blocks. Third, quadratic models necessarily predict ever-increasing or ever-decreasing responses – an unrealistic property as responses to selection, although they might become null, can never become negative (*i.e.* in a direction opposite to selection).

Using piecewise regressions, we observed that the response to selection differed before and after the BP, and we therefore considered independently the interactions among the fixed factors before and after this point. The value of the BP was estimated together with the regression model using a maximum-likelihood procedure coded in R (code in *http://stats.stackexchange.com/questions/19772/estimating-the-break-point-in-a-broken-stick-piecewise-linear-model-with-random-effects-in-R*). At each generation the selection differential was estimated as the difference between the mean of (W/L) in selected individuals and all measured individuals. The regression analysis was repeated using the cumulative selection differential (CSD) instead of generations, and we also regressed the CSD on the number of generations of selection. In both analyses, we introduced a breakpoint at generation 3, based on the previous regression (the breakpoint was estimated at generation 2.81).

We also used a regression analysis to estimate the average realized heritability over the whole treatment. Under the outcrossing treatment the realized heritability (h^2) can indeed be derived as the slope of cumulative response over cumulated CSD averaged over the whole duration of the experiment. In the selfing treatment, this slope represents a peculiar form of broad-sense heritability (H^2), which differs from narrow-sense h^2 as it includes variable amounts of dominance and interaction variance. Nevertheless, irrespective of underlying

genetics, this slope remains indicative of how efficient selection is at generating a phenotypic response in the population, on average.

We also monitored the evolution of the phenotypic variance (represented as its square root, phenotypic standard deviation) across generations. It was analyzed using a regression model similar to the ones presented above, with generation, line and treatment as fixed factors, and line replicate as a random factor. A BP was enforced at generation 3.

All comparisons of the shell trait were conducted using chi-square likelihood-ratio tests computed using restricted maximum likelihood for random terms and maximum likelihood for fixed effects. All analyses were performed with R version 3.0.0 packages lme4 (Bates et al. 2013).

Microsatellite Variation. The analysis was done as described in Sourrouille et al. (2003) andEscobar et al. (2008). DNA was extracted from 40 / 50 individuals per line, replicate and treatment at the G0 / G6 generations respectively. We initially genotyped 10 loci and then reduced to 7 loci that were both repeatable and polymorphic (AF108762, AF10874, Pac1, Pac2, Pasu1-2, Pasu1-9, Pasu1-11). Gene diversity and the observed heterozygosity were estimated using Genetix 4.2 (Belkhir et al. 2004).

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Table 1. **Regression of individual shell roundness on time (generations) for lines and treatments.**

The individual phenotypic values are regressed on the number of selection generations, and the factors are experimental evolution line type (C and S) and treatment (outcrossing or selfing). We consider all interactions. Line type, treatment and their interactions are modelled as fixed effects. As a breakpoint (BP) in the response to selection was detected (see Fig. 1A), the generation parameter is considered both before and after the BP. Replicate refers to replicate line within each type (2 per type) and block to temporal periods of one month (21) that are modelled as random effects.The values reported are Chi-2 and the probability associated to Likelihood ratio tests (one degree of freedom in all tests).

Table 2.

Effect	**Chi-2**	***P***
Generation before BP	51.53	$7\ 10^{-13}$
Generation after BP	70.64	$< 2\ 10^{-16}$
Line type * generation before BP	5.95	0.015
Treatment * generation before BP	23.59	$1.2\ 10^{-6}$
Line type * generation after BP	28.99	$7.3\ 10^{-8}$
Treatment * generation after BP	5.59	0.018
Line type* treatment * generation before BP	2.63	0.104
Line type * treatment * generation after BP	1.43	0.231
Replicate	1539.9	$< 2\ 10^{-16}$
Block	390.4	$< 2\ 10^{-16}$

Regression of individual shell roundness on cumulative selection differential for lines and treatments.

The phenotypic values are regressed on cumulative selection differential (CSD), and the factors are experimental evolution line type (C and S) and treatment (outcrossing or selfing). The breakpoint is not estimated, but enforced at three generations (see Fig. 1B) for comparison with model results in Table 1. Legend as in Table 1. The number of degrees of freedom is one in all tests, expect in the two interactions involving replicate (df = 3).

Effect	**Chi-2**	***P***
CSD before BP	35.51	$2\ 10^{-9}$
CSD after BP	20.61	$5.6\ 10^{-6}$
Line type * CSD before BP	16.91	$3.9\ 10^{-5}$
Treatment * CSD before BP	11.75	$6\ 10^{-4}$
Line type * CSD after BP	0.08	0.773
Treatment * CSD after BP	17.74	$1.5\ 10^{-5}$
Line type * treatment * CSD before BP	7.21	0.007
Line type * treatment * CSD after BP	2.99	0.084
Replicate * CSD before BP	7.50	0.032
Replicate * CSD after BP	31.93	$2.8\ 10^{-7}$
Block	397.1	$< 2\ 10^{-16}$

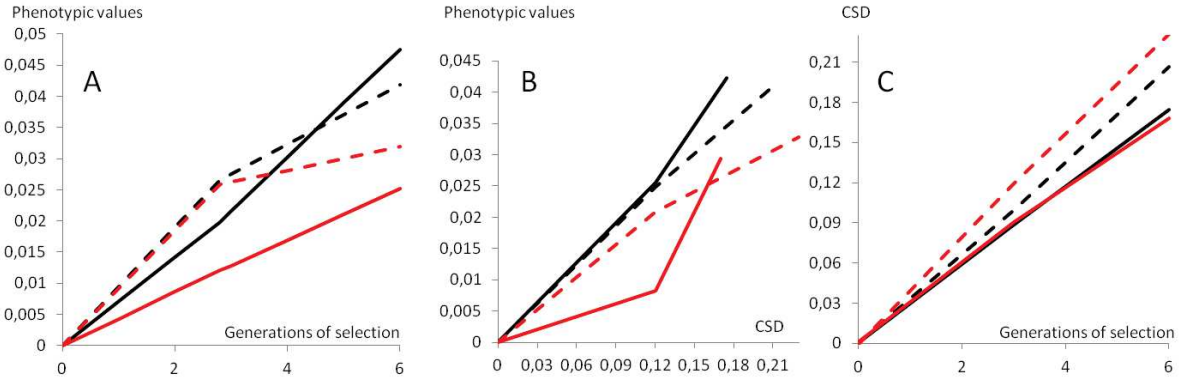
Table 3. **Regression of cumulative selection differential on time (generations) for lines and treatments.**

The cumulative selection differential (CSD) is regressed on the number of selection generations, and the factors are experimental evolution line (C and S) and treatment (outcrossing or selfing). The breakpoint is not estimated, but enforced at three generations (see Fig. 1C) for comparison with model results in Table 1. Legend as in Tables 1 and 2.

Effect	**Chi-2**	***P***
Generation before BP	987.7	$< 2\ 10^{-16}$
Generation after BP	2229.1	$< 2\ 10^{-16}$
Line * generation before BP	3.11	0.078
Treatment * generation before BP	36.08	$1.9\ 10^{-9}$
Line * generation after BP	0.25	0.615
Treatment * generation after BP	46.7	$8.4\ 10^{-12}$
Line * treatment * generation before BP	7.82	0.005
Line * treatment * generation after BP	2.46	0.117
Replicate * generation after BP	9.16	0.027

Figure legends

Fig. 1. *(A)* Regression of individual shell roundness on time (number of selection generations) for lines and treatments. A breakpoint is estimated at 2.81 generations (95% CI: 2.70 - 3.11). Significance of effects evaluated in Table 1. *(B)* Regression of individual shell roundness on cumulative selection differential for lines and treatments. Significance of effects evaluated in Table 2. A breakpoint is enforced at generation 3 (see Table 2). *(C)* Regression of cumulative selection differential on time (generations) for lines and treatments. A breakpoint is enforced at generation 3 but is not visible on Figure. Significance of effects evaluated in Table 3. C/Out: black continuous; C/Self: black dotted; S/Out: red continuous; S/Self: red dotted.



Experimental evidence for the long-term negative effects of selfing

Elsa Noël, Philippe Jarne, Sylvain Glémin, Alicia MacKenzie, Adeline Segard, Violette Sarda, and Patrice David

Supporting Information Appendix

Figure S1. Mean phenotypic response (obtained by subtracting the mean of the initial generation to data points) and their standard error per line (C: black; S: red), replicate per line (C1: square; C2: triangle; S1: circle; S2: diamond), and treatment (outcrossing: continuous lines; selfing: dotted lines). Artificial selection begins between the second and third point for each line as there is one generation to install the reproductive treatment before the beginning of the selection).

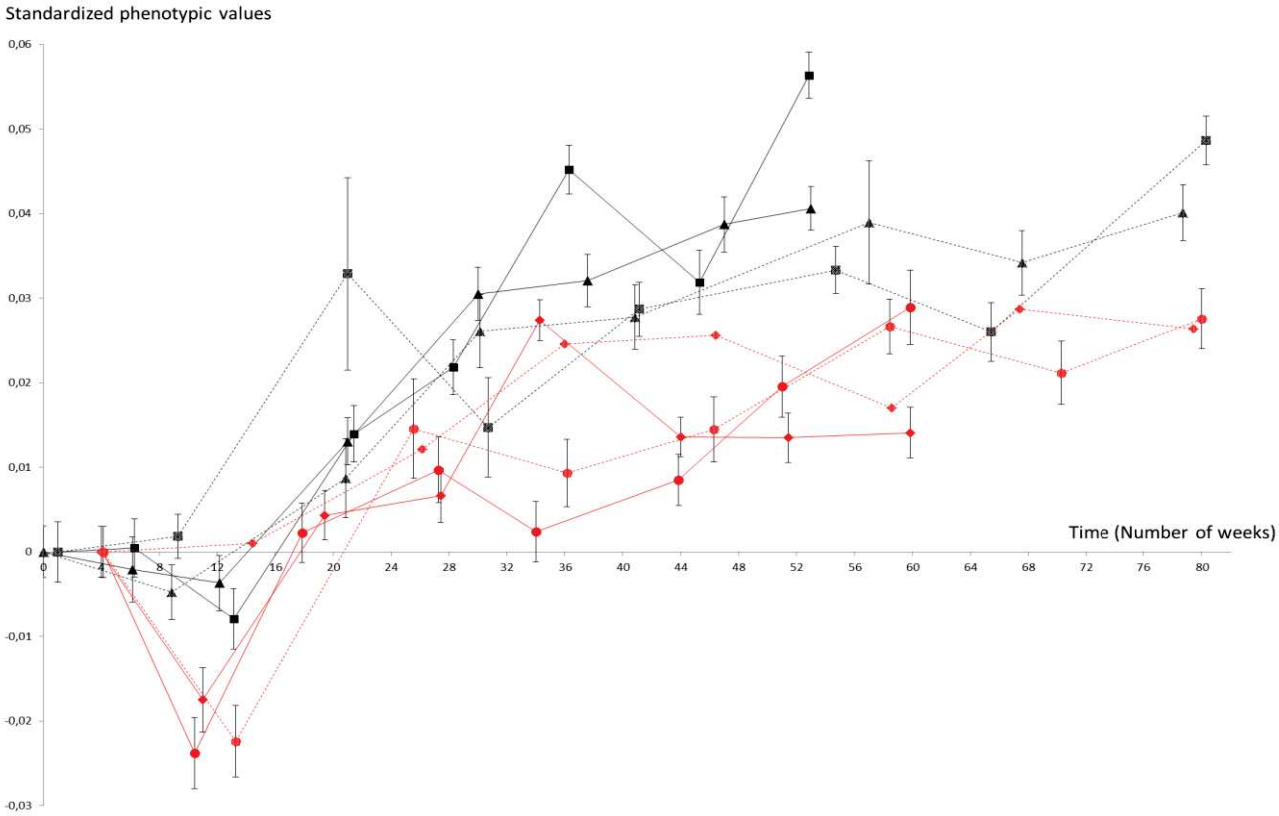


Figure S2. Regression of the cumulative response to selection on the cumulative selection differential (CSD) for lines and treatments. C/Out: black continuous; C/Self: black dotted; S/Out: red continuous; S/Self: red dotted. Significance of effects evaluated in Table S1. The same data are modeled in Fig. 1B and Table 2 with a breakpoint. The current analysis is run to obtain mean heritabilities. Note that the strength of selection can slightly vary with generations because both the intensity of selection and the variance upon which selection is applied vary, resulting in a larger cumulative strength of selection in the selfing than in the outcrossing treatment.

Comment: the slope of the current regression gives the mean realized heritability over generations. It is 0.304 in C/Out, i.e. much larger than in C/S (0.198). The almost significant 'line x CSD' interaction (Table S1) suggests a realized heritability of 0.230 for S/Out individuals.

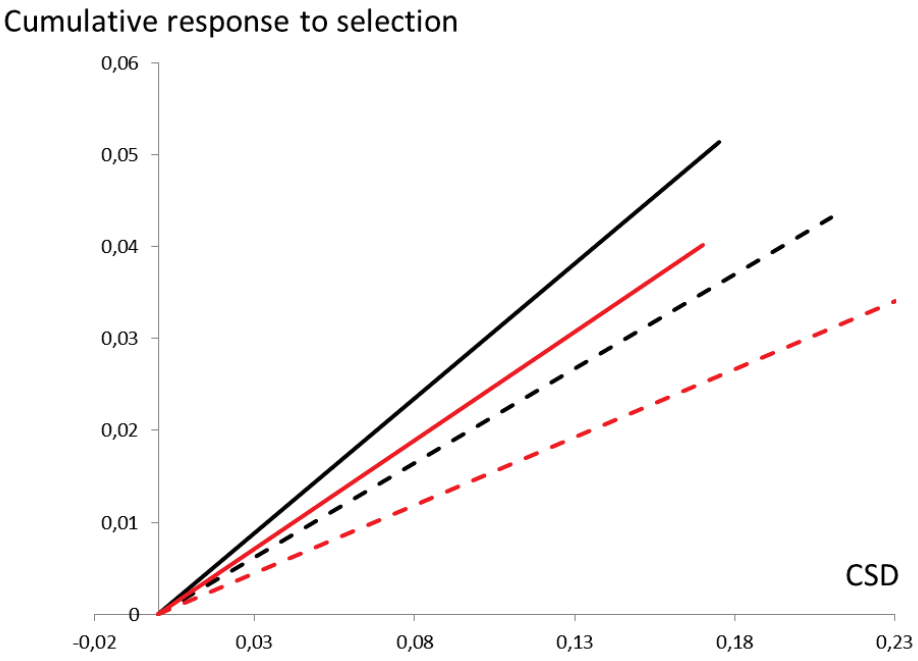


Figure S3. Regression (fitted values) of the standard deviation of shell roundness on the number of generations of selection for lines and treatments. A threshold point in the response to selection is enforced at generation 3. C/Out: black continuous; C/Self: black dotted; S/Out: red continuous; S/Self: red dotted. See Table S2 for an analysis of effect significance.

Comment: as mentioned in main text, the increase in cumulative selection differential with generations reflects the phenotypic variance within populations, requiring to monitor the variation in phenotypic standard deviation (SD) over generations. Confirming our previous observations, the SD is consistently higher in selfing than in outcrossing treatments throughout the experiment. Although there is no consistent temporal pattern, the phenotypic SD tend to increase before the breakpoint in selfing populations and then decrease. The reverse pattern is observed in the S/Out line and a consistent decrease in the C/Out line.

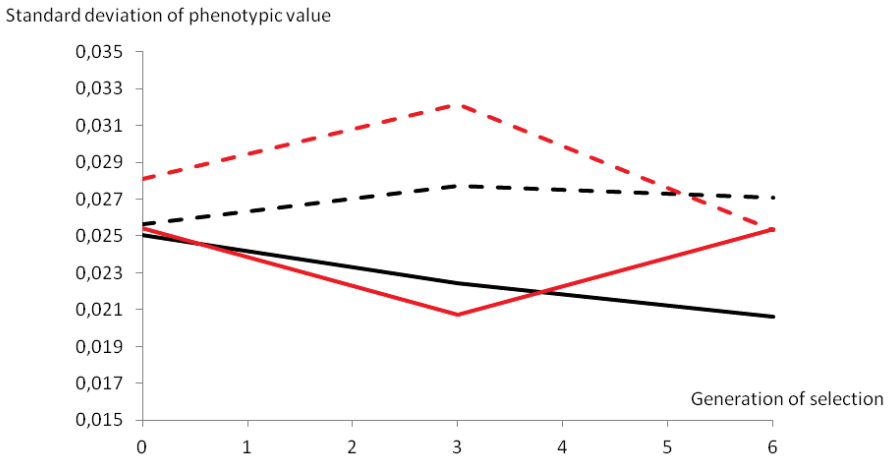


Figure S4. Distribution of the phenotypic variance per generation of selection, line, replicate (per line) and treatment. C/Out (line/treatment): black continuous; C/Self: red dotted; S/Out: red continuous; S/Self: black dotted. 1 and 2 refer to the two replicates per line.

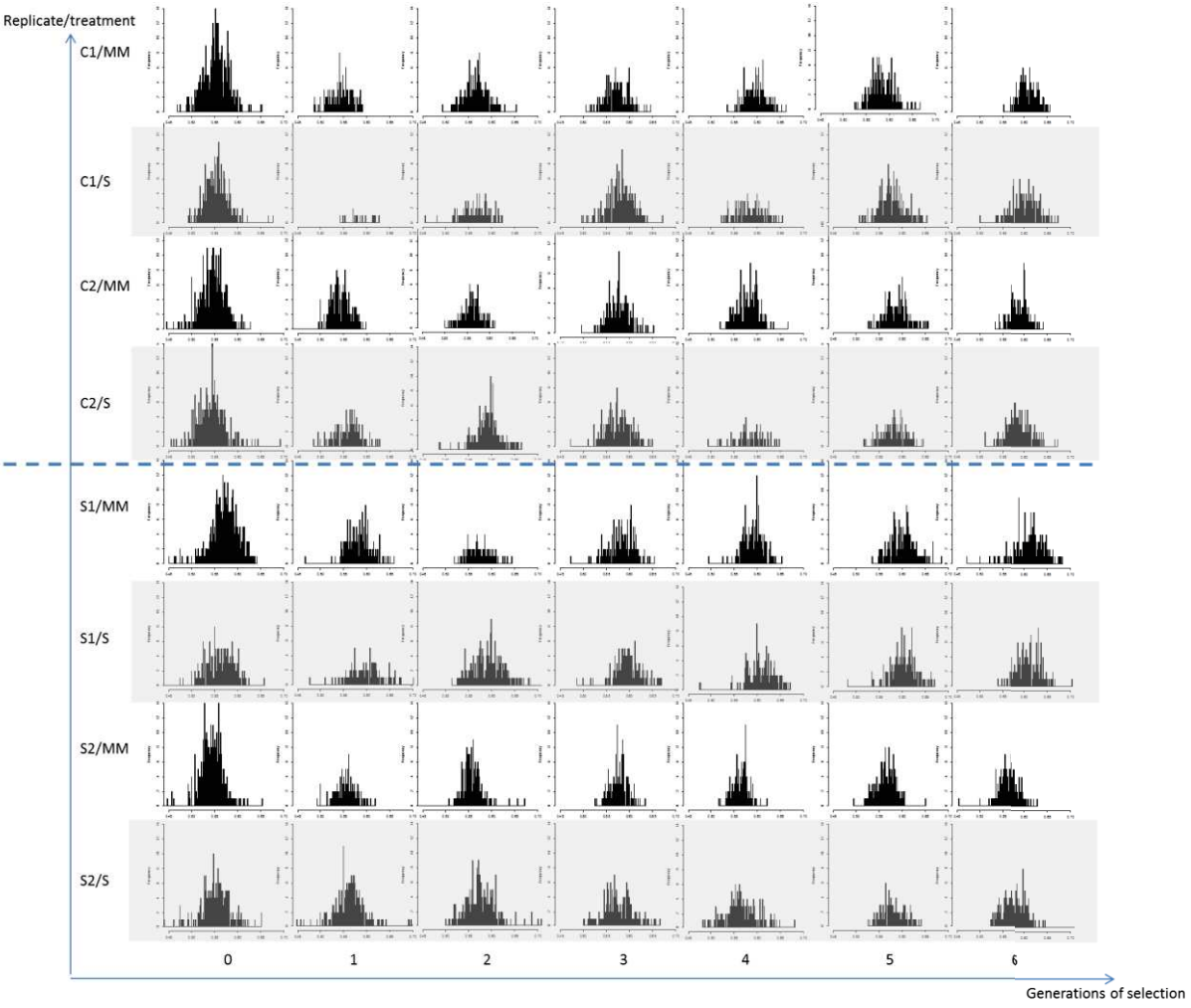


Fig. S5. Genetic diversity before the initiation of selection (G0) and after six generations of selection (G6) per line, treatment and replicate. Shaded bars are gene diversity (expected heterozygosity under Hardy-Weinberg equilibrium) and open bars denote observed heterozygosity. C/Out (line/treatment): black continuous; C/Self: red dotted; S/Out: red continuous; S/Self: black dotted. 1 and 2 refer to the two replicates per line.

Comment: The observed heterozygosity is initially lower in selfing than in outcrossing lines, and as expected very low after six generations of selfing. However the reduction in heterozygosity (compared to outcrossing counterparts) is not as drastic as expected under neutrality (*i.e.* by a factor 2 or 2^7 for the G0 and G6 respectively – recall that lines / replicates experiencing the selfing treatment went through one generation of selfing before the initiation of selection).

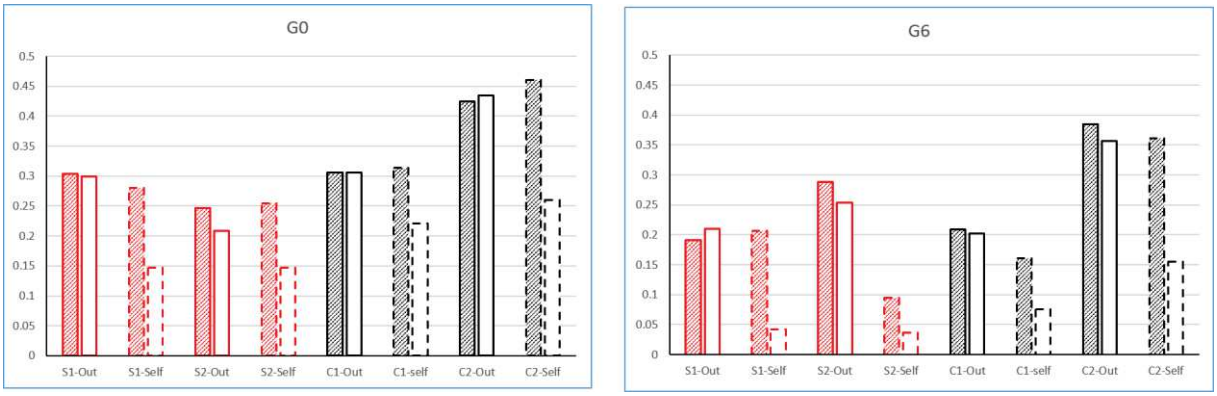


Table S1. **Regression of shell roundness on the cumulative selection differential (CSD) for lines and treatments.**

The mean phenotypic values are regressed on the CSD, and the factors are experimental evolution line (C and S) and treatment (outcrossing or selfing). We consider all interactions. Line, treatment and their interactions are modelled as fixed effects. Replicate refers to line replicate (2 per line) and block to temporal periods of one month (21) that are modelled as random effects. The values reported are Chi-2 and the probability associated to Likelihood ratio tests (one degree of freedom in all tests). See Fig. S2 for a representation of regressions.

Effect	**Chi-2**	**P**
CSD	170.59	$< 2\ 10^{-16}$
Line * CSD	3.00	0.083
Treatment * CSD	7.62	0.006
Line * Treatment * CSD	0.18	0.670
Replicate	60.13	$8.9\ 10^{-15}$

Table S2. Regression of the standard deviation of shell roundness on the number of generations of selection for lines and treatments.

The standard deviation of shell roundness is regressed on generations, and the factors are experimental evolution line (C and S) and treatment (outcrossing or selfing). We consider all interactions. Line, treatment and their interactions are modelled as fixed effects. A threshold point in the response to selection is enforced at generation 3, and the generation parameter is considered before (generation before BP) and after (generation after BP) the threshold. Legend as in Fig. S1. See Fig. S3 for a representation of regressions.

Effect	**Chi-2**	**P**
Generation before BP	0.10	0.751
Generation after BP	1.08	0.298
Line	3.05	0.081
Treatment	51.24	$8\ 10^{-13}$
Line * generation before BP	0.00	0.970
Treatment * generation before BP	12.63	0.0003
Line * generation after BP	0.00	0.947
Treatment *Line	1.23	0.267
Treatment * generation after BP	5.43	0.020
Line * treatment * generation before BP	1.17	0.279
Line * treatment * generation after BP	8.18	0.004

Conclusion générale de la thèse

L'évolution de l'autofécondation et de ses conséquences est une « vieille » question en biologie évolutive, puisqu'elle a été abordée par Darwin (1876), et a fait l'objet plus récemment de contributions importantes (Fisher 1941, Baker 1967, Jain 1976). Les articles de Lande & Schemske (1985) et de Charlesworth & Charlesworth (1987) ont donné une nouvelle vigueur à ce champ d'analyse en mettant l'accent sur les aspects génétiques de cette évolution, en particulier la dépression de consanguinité. Parallèlement, les travaux de D. Lloyd, de S. Barrett et de leurs collaborateurs (Lloyd 1979, 1992, Lloyd & Schoen 1992, Barrett et al. 1996, Barrett 2002) ont placé l'emphase sur des aspects plus phénotypiques, en particulier la phénologie de reproduction (par ex., les notions d'autofécondation prioritaire, compétitive et retardée) et divers polymorphismes sexuels. Dans ces perspectives génétique et/ou écologique, un nombre considérable d'études a été publié depuis lors, en particulier chez les plantes (voir les revues de Goodwillie et al. 2005, Johnston et al. 2009, Pannell et al. 2015), mais aussi chez les animaux (Jarne & Auld 2006, Escobar et al. 2011). Il en résulte une meilleure connaissance sous bien des aspects. Par exemple, la distribution des taux d'autofécondation présente une distribution en U, en particulier chez les animaux pour lesquels on a pu mettre de côté des artefacts d'estimation de taux d'autofécondation (Escobar et al. 2011). On connait aussi assez bien la relation entre taux d'autofécondation et dépression de consanguinité (Husband & Schemske 1996, Goodwillie et al. 2005, Escobar et al. 2011). Si on s'en tient au cas des animaux, on sait aussi que les taux d'autofécondation faibles sont associés à des traits qui permettent une résistance à l'autofécondation (par ex., temps d'attente).

Les processus sélectifs agissant sur le système de reproduction et les traits associés ont quant à eux fait l'objet d'approches plus « phénotypiques » que génétiques – peut-être en raison de l'emphase mise sur la question de la dépression de consanguinité. On peut en particulier noter que les approches par génétique quantitative ont été moins fréquentes – on peut tout de même citer pour exemple les travaux de Ashman & Morgan (2004) et de Kelly (2005). Il en résulte qu'on connait encore mal la relation entre sélection, système de reproduction, dépression de consanguinité et traits (voir par ex. Charlesworth & Charlesworth 1995, Lande & Porcher 2015). Peut-être plus surprenant est le fait que la méthodologie dite d'évolution expérimentale n'a été que marginalement utilisée dans le contexte de l'évolution de l'autofécondation, et peut-être plus souvent chez les animaux (Morran 2009, Chelo et al. 2014) que chez les plantes (Mazer 1999, Bobdyl Roels & Kelly 2011). Or il y a là un potentiel important pour évaluer en temps réel la plupart des questions liées à l'autofécondation et son évolution – avec bien entendu toutes les limitations d'une approche au laboratoire et donc quelque peu artificielle. C'est dans ce contexte que se situe ce travail de thèse qui apporte, je l'espère, quelques éclaircissements concernant divers aspects cités plus haut, en particulier sur les conséquences génétiques et phénotypiques de l'évolution de taux d'autofécondation élevés et sur la réponse à la sélection en régime d'autofécondation.

A propos de dépression, de temps d'attente et de taux d'autofécondation

Tirant partie de lignées expérimentales de *P. acuta* mise en place depuis une vingtaine de générations, l'expérience exposée dans le chapitre II montre qu'une partie de la dépression de consanguinité peut être purgée facilement et en peu de générations via un régime de reproduction dans lequel l'autofécondation est pratiquée une génération sur deux. Il est notable qu'une partie ne l'est pas, mais cela est en phase avec le fait que les espèces fortement autofécondantes maintiennent une dépression de consanguinité (Husband & Schemske 1996,

Escobar et al. 2011). Cette purge se déroule conjointement à une diminution du temps d'attente, donc un recours plus rapide à l'autofécondation. Une telle relation entre dépression et temps d'attente a déjà été observé chez *P. acuta* entre familles et entre populations (Tsitrone et al. 2003b, Escobar et al. 2007, 2009), mais aussi dans le groupe des Hygrophila (Escobar et al. 2011). Cette coévolution pose le problème de « l'œuf et de la poule » : on ne sait pas si c'est la diminution du temps d'attente qui facilite l'autofécondation et qui entraîne donc la purge des mutations délétères, ou si c'est cette purge qui rend l'autofécondation moins coûteuse et qui fait donc diminuer le temps d'attente (on peut imaginer que les deux processus fonctionnent conjointement).

Dans notre expérience, l'autofécondation ne peut pas, au moins initialement, être considérée comme une conséquence de la purge, puisqu'elle est imposée par l'expérimentateur à tous les individus indépendamment de leur fardeau de mutations délétères et de leur temps d'attente. On peut simplement noter que cela mime des conditions de densité de population très faible contraignant le recours à l'autofécondation – on se place dans les conditions où l'autofécondation est utilisée comme assurance de reproduction (Baker 1955, Jarne & Charlesworth 1993). Nous avons montré que cette autofécondation imposée suffit à enclencher la purge et l'évolution du temps d'attente, établissant ainsi une « preuve de possibilité » d'un scénario d'évolution dont le départ est précisément lié à des conditions adverses requérant une telle assurance. Cependant, nos conditions expérimentales diffèrent d'un scénario naturel dans le sens où nous avons, par un régime extrême d'alternance entre 100% d'autofécondation forcée et 100% de reproduction en couples, supprimé toute possibilité de mise en place d'une corrélation génétique entre temps d'attente et dépression de consanguinité au sein des populations telle que celle observée entre populations naturelles, voire entre familles, de *P. acuta* (Escobar et al. 2009). Les différences de temps d'attente sont

alors sans effet exprimé sur le taux d'autofécondation puisque celui-ci ne dépend que de l'expérimentateur. Nous montrons donc que le temps d'attente et la purge peuvent évoluer conjointement en réponse à la même situation (un manque périodique de partenaires) même s'ils n'ont pas l'opportunité d'agir l'un sur l'autre. Dans la nature, la réduction de la disponibilité en partenaires se produit régulièrement chez *P. acuta* (Henry et al. 2005), et plus largement chez les Hygrophila (voir Brown 1994, Dillon 2000). Elle est cependant probablement moins forte, et en tous cas plus imprévisible, que dans notre protocole. L'évolution conjointe de la dépression et du temps d'attente peut alors être renforcée par une boucle de rétroaction positive entre ces caractères (conformément aux corrélations génétiques observées en populations naturelles ; Escobar et al. 2009).

Il est important de noter que nous n'avons pas vérifié si le taux d'autofécondation « naturel » avait commencé à augmenter dans nos lignées expérimentales, en d'autres termes si on tendait à évoluer d'une autofécondation retardée vers une autofécondation prioritaire. L'utilisation de la méthode de « progeny-arrays », obtenus en croisements libres, et de marqueurs moléculaires (Jarne & David 2008) permettrait de quantifier le taux d'autofécondation. La valeur mesurée du temps d'attente (7 jours) entre pour le moment dans la variation observée dans 26 populations de *P. acuta* (Escobar et al. 2009). Il est probable qu'il soit nécessaire de mener l'expérimentation pendant encore de nombreuses générations avant de voir apparaître une autofécondation prioritaire (les lignées expérimentales sont actuellement en G50 environ). Le temps d'attente peut se réduire à zéro, mais ce n'est pas encore suffisant pour arriver à cette autofécondation préférentielle, donc on n'a fait que la moitié du chemin donné dans notre scénario initial et il faut continuer l'évolution expérimentale. On peut aussi envisager d'extraire une partie des individus des lignées S afin de les replacer en situation d'allofécondation. Cela permettrait de tester si le temps d'attente

ré-augmente, ce qui indiquerait la possibilité d'une évolution à rebours, et aussi de mesurer le temps requis pour ré-accumuler de la dépression. Ce temps est probablement très long si on ne compte que sur l'apparition de mutations fortement délétères, mais qui peut significativement se réduire si on considère que des effets épigénétiques participent à cette dépression (voir ci-dessous).

On attendait mieux de l'allocation des ressources

L'expérience mentionnée ci-avant visait aussi à évaluer si l'allocation des ressources pouvait évoluer sous un régime d'autofécondation fréquente. On attendait en particulier une réallocation des ressources de la fonction mâle vers la fonction femelle, comme prédit par la théorie (Charlesworth & Charlesworth 1981, Charnov 1982) et observé par analyses comparatives (par ex., ratio pollen/ovule chez les plantes, Cruden 1977). Ces observations sont cependant faites dans des espèces qui ont réalisé la transition vers l'autofécondation depuis des milliers de générations, et il est difficile de savoir à quel moment a lieu cette réallocation.

L'approche par évolution expérimentale permet cependant d'aborder cette question. Nous n'avons pas observé de telle réallocation, ce à quoi on peut donner plusieurs explications : (i) il s'agit d'une évolution consécutive à l'évolution vers des taux d'autofécondation élevé et sur une plage de temps beaucoup plus longue que celle de notre expérience. Seule une nouvelle évaluation, menée après un plus grand nombre de générations d'évolution, pourrait permettre d'apporter des éléments de réponse. (ii) Les allèles à effets antagonistes nécessaires à cette évolution n'étaient pas présents dans nos lignées expérimentales, et il serait donc nécessaire d'attendre l'apparition de telles mutations – ce qui prendrait un temps considérable, excédant peut-être ce qui est envisageable avec un

organisme dont le temps de génération est de deux-trois mois environ. (iii) La diminution de la pression de sélection sur la fonction mâle dans notre protocole en régime d'autofécondation (*i.e.* absence de compétition post-copulatoire et dans une certaine mesure pré-copulatoire) est importante, mais pas complète. En effet, les individus sont appariés une génération sur deux, et sont quand même en compétition avec leur partenaire pour la fonction mâle. (iv) L'étude des corrélations entre valeur sélective mâle et femelle dans les populations contrôles pourrait apporter des éléments de réponse. Dans une expérience réalisée par T. Jänicke en 2015 (données non publiées), on a relevé une corrélation génétique positive entre ces valeurs sélectives dans une population naturelle de *P. acuta* proche de Montpellier, les deux fonctions étant positivement corrélées à la taille. Si la valeur sélective de la fonction mâle est positivement corrélée à la valeur sélective de la fonction femelle, il est beaucoup plus long de détruire cette association d'allèles et permettre une réallocation, en particulier avec un déséquilibre de liaison conservé par l'autofécondation une génération sur deux. (v) S'il n'est pas douteux qu'une fonction (ici, mâle) inutile et coûteuse est contre-sélectionnée, sa disparition ne va pas nécessairement bénéficier à l'autre fonction (ici, femelle) – la réallocation peut avoir lieu vers d'autres fonctions (Charnov 1982). On peut envisager dans notre protocole expérimental, puisqu'une partie de la dépression a été purgée et que les lignées sont moins fragiles, d'augmenter la pression sur l'autofécondation en ne présentant des partenaires aux lignées S qu'une génération sur 4, afin de tester si l'ensemble des traits composant le syndrome d'autofécondation évoluent plus vite. Il serait d'ores et déjà possible d'utiliser les lignées S /Self de notre seconde expérience – celle-ci se terminait après six générations d'autofécondation, mais trois générations de plus ont maintenant été réalisées, pour mesurer à nouveau les mêmes traits (dépression, temps d'attente et allocation mâle/femelle) en supposant que la forme de la coquille n'est pas corrélée au syndrome

d'autofécondation et que la sélection artificielle appliquée à la coquille n'a pas eu d'effets sur l'évolution de ce syndrome.

Consanguins …. et pas très répondants à la sélection

La partie la plus conséquente de mon travail de thèse a porté sur l'évaluation de la relation entre ampleur de la réponse à la sélection et système de reproduction. Il s'agit d'un champ qui a été peu travaillé d'un point de vue à la fois expérimental (voir la revue de Charlesworth & Charlesworth 1995 – peu de travaux ont été produit depuis) et théorique (Charlesworth & Charlesworth 1995, Lande & Porcher 2015), si on compare à la montagne d'études portant sur la dépression de consanguinité. Mon expérimentation, qui a duré 21 mois, a permis de montrer qu'un régime autofécondant affectait négativement la réponse à la sélection par rapport à un régime allofécondant. Par ailleurs, cette réponse est aussi largement affectée par le régime de reproduction pratiqué antérieurement. L'autofécondation passée diminue, comme attendu (Charlesworth & Charlesworth 1995, Lande & Porcher 2015), la variabilité disponible sur laquelle la sélection peut agir, et par conséquent la réponse à la sélection. On a donc bien un moindre potentiel adaptatif dans les populations qui ont pratiqué régulièrement l'autofécondation. Quel que soit l'historique de reproduction, autofécondant ou allofécondant, notre travail indique aussi qu'une population répond tout d'abord mieux à la sélection en autofécondation qu'en autofécondation, comme attendu sous l'hypothèse d'augmentation de variance phénotypique et le placement d'allèles à l'état homozygote (Wright 1921, Charlesworth & Charlesworth 1995, Lande & Porcher 2015). Cependant, cet avantage s'estompe dans notre expérience après seulement trois générations de sélection. Cela est probablement dû à des interférences sélectives.

En quoi cela est-il intéressant pour comprendre la dynamique de transition entre autofécondation et allofécondation ? Si l'on considère une population allofécondante qui doit répondre à une pression de sélection sur une courte durée (par ex, une à deux générations pour atteindre le nouvel optimum), une reproduction par autofécondation donnera un avantage immédiat en termes de variance disponible, mais le faible nombre de générations pendant lequel ceci reste valable ne permet pas une totale transition de l'allofécondation vers l'autofécondation. Il serait pourtant optimal de pouvoir commuter de l'autofécondation (sur une courte période pour une adaptation rapide à un optimum proche) vers l'allofécondation (une fois le nouvel optimum atteint). On s'attendrait donc à un avantage aux régimes plastiques, c'est-à-dire que la sélection agirait sur la pente de la norme de réaction du système de reproduction (*i.e.* la meilleure amplitude de variation du taux d'autofécondation en fonction des conditions environnementales) et sélectionnerait les individus les plus plastiques. Une telle plasticité aboutirait à des taux d'autofécondation variables et non systématiquement extrêmes dans la nature, et seraient classifiés comme « régimes mixtes ». Cependant les systèmes mixtes habituellement considérés dans la théorie des systèmes de reproduction sont des stratégies où l'on autoféconde systématiquement une part des ovules et non des stratégies plastiques où cette part dépend des conditions. Certains modèles prédisent effectivement qu'un régime mixte (non plastique) est avantageux en raison d'une augmentation de la variance phénotypique avec le placement des allèles à l'état homozygote (Glémin & Ronfort 2013, Hartfield & Glémin 2014), mais cet avantage à long terme pour la population ne garantit pas un avantage individuel immédiat permettant à une stratégie mixte d'envahir la population dans laquelle elle réside. Il en résulte que les conditions environnementales devraient être bien spécifiques pour permettre à une telle stratégie d'envahir. Chez les animaux, les régimes mixtes semblent assez rares (voir Jarne & Auld 2006, Escobar et al. 2011). On ne sait pas si ces régimes de reproduction tranchés entre espèces sont liés à des

conditions de vie très différentes, ou s'il existe de réelles contraintes comme peut l'être la géitonogamie chez les plantes (voir plus bas). Il est à noter que si la pression de sélection mentionnée plus haut perdure et/ou si le nouvel optimum est assez éloigné du point de départ, l'adaptation d'une population autofécondante sera difficile, voire impossible, pour des raisons démographiques : si la population passe en dessous d'un seuil démographique fatidique, elle est dans tous les cas vouée à l'extinction (Lande, 1987). Dans ce cas, l'allofécondation reste avantagée.

Et des résultats à approfondir

Notre travail a mis à jour quelques résultats surprenants qui mériteraient d'être analysés plus avant, et de nouvelles pistes à explorer. J'en citerai trois.

L'architecture de la dépression de consanguinité ne semble pas être simplement constituée d'allèles à hérédité mendélienne et. Il est possible qu'elle dépende aussi d'interactions « génétique x environnement » ou d'effets épigénétiques comme les méthylations (Vergeer et al. 2012). La curieuse dépression négative mesurée dans un des réplicats de la lignée S semble aller dans ce sens. Une nouvelle mesure de cette dépression est tout d'abord prévue afin de confirmer cette tendance. L'évaluation de l'influence d'effets maternels sur la valeur sélective moyenne est envisageable, par exemple en utilisant des croisements plein-frères d'autofécondation (puisqu'on ne peut pas mesurer d'effets maternels sur des descendants d'autofécondation). Une autre piste est l'analyse de mutations chromosomiques chez des individus consanguins, généralement sous-dominantes (Templeton 1981) et pouvant générer une dépression négative. L'occurrence de telles mutations est très mal connue chez les gastéropodes en général.

Un deuxième résultat qui mérite plus d'attention est l'évolution de la diversité neutre dans les lignées expérimentales, mais aussi pendant l'expérience de sélection sur la forme de la coquille. En particulier, les lignées ont perdu beaucoup moins d'hétérozygotie observée lors de cette expérience que ce qui est prévu après plusieurs générations d'autofécondation. Une explication pourrait être que le fardeau est réparti en de nombreux points du génome, ce qui rend difficile le placement des génomes à l'état homozygote, une forme de superdominance associative à cause de la liaison sur l'ensemble du génome. Si c'est le cas, un approfondissement, serait nécessaire pour estimer avec un protocole adapté la perte réelle d'hétérozygotie sur les marqueurs neutres. La perspective d'utiliser les méthodes récentes basées sur les NGS (de type Rad-Seq, Baird et al. 2008) pour avoir rapidement de nombreux marqueurs densément répartis dans le génome est probablement prometteuse de ce point de vue. Une modélisation du processus expérimental permettrait aussi de mieux comprendre l'évolution attendue de la diversité.

La piste à explorer qui me parait la plus intéressante est celle d'intégrer des paramètres démographiques à la réflexion sur les avantages à court et moyen termes de l'autofécondation. En effet, dans notre expérience, on a fait en sorte que les lignées pratiquant l'autofécondation une génération sur deux (première expérience) ou systématiquement (deuxième expérience) soient maintenus à des effectifs fixes. Cependant, de telles populations ne manqueraient pas de subir des pertes de lignées en conditions naturelles, ce qui pourrait amener en dessous d'un seuil démographique et conduire à l'extinction de la population (Lande 1987). On associe souvent l'autofécondation majoritaire avec une stratégie de colonisation (Price & Jain 1981; Pannell et al. 2015), ce qui semble indiquer que la densité est un paramètre important. Cette dernière est impliquée dans l'assurance de reproduction et un régime de reproduction par autofécondation. On notera aussi que la dépression de consanguinité biparentale en faible

densité peut déplacer la balance entre dépression de consanguinité et avantage génétique automatique de l'autofécondation (Uyenoyama 1986). Cependant, les données sur l'association entre régime de reproduction et mode de vie sont rares chez les animaux. Une revue de la littérature afin d'estimer la réelle corrélation entre système de reproduction et dynamique de colonisation est donc nécessaire. Cependant, *P. acuta* est une espèce invasive, mais présente pourtant un système de reproduction préférentiellement allogame. Elle a réussi à coloniser une large gamme d'habitats, de l'équateur aux cercles polaires ; par ailleurs, elle semble être une compétitrice efficace lorsqu'elle envahit des milieux occupés par la conspécifique *Aplexa marmorata*, une espèce de physe autofécondante proche phylogénétiquement de *P. acuta*. C'est le cas par exemple dans la métacommunauté d'eau douce de Guadeloupe (Chapuis et al. soumis). Il est possible que les avantages de l'autofécondation soient réservés aux situations de colonisation sans compétition interspécifique, et que cet avantage disparaisse si une espèce compétitive allofécondante arrive ensuite (comme c'est le cas en Guadeloupe).

De plus, les associations colonisation / autofécondation sont souvent formulées chez les plantes, dont le taux d'autofécondation « stratégique » est difficile à distinguer du taux d'autofécondation inévitable par géitonogamie, ce qui surestime finalement ce taux. Les plantes cléistogames sont pour cela un bon modèle puisqu'on peut considérer le taux d'autofécondation des fleurs ouvertes comme de la géitonogamie et celui des fleurs fermées comme une vraie stratégie de reproduction. (Culley & Klooster, 2007) On pourrait envisager une expérience de grande ampleur comparant des espèces animales et/ou végétales, en manipulant le système de reproduction et la compétition pour la ressource.

En conclusion, l'étude de l'évolution de l'autofécondation et de ses conséquences s'est révélé être un champ de recherche fertile et stimulant. De nombreuses pistes doivent encore être explorées, faisant appel à un mélange d'approches expérimentales, théoriques, phylogénétiques ou comparatives, pour en parfaire notre compréhension ; beaucoup plus, probablement, que Darwin ne l'avait imaginé en lançant ce champ de travail il y a déjà 150 ans.

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Résumé : Une grande partie des organismes hermaphrodites, qu'il s'agisse de plantes ou d'animaux, est capable de se reproduire par autofécondation, comme alternative à la fécondation croisée. Or les modèles théoriques prédisent un ensemble de conséquences évolutives importantes liées à l'autofécondation. La première prédiction est qu'une population pratiquant l'autofécondation est moins sensible à la dépression de consanguinité qu'une population à reproduction croisée, car une partie de la dépression a été « purgée », c'est-à-dire que les allèles délétères récessifs sont éliminés par la sélection naturelle plus facilement en autofécondation. Cette purge entraine en retour une sélection positive sur l'autofécondation. On attend aussi chez ces populations l'évolution de traits facilitant l'autofécondation (par ex., des fleurs fermées), ainsi qu'une réallocation de ressources de la fonction mâle vers la fonction femelle, en raison d'une sélection sexuelle réduite sur la fonction mâle.Une reproduction par autofécondation va aussi considérablement affecter la variabilité disponible en raison d'une taille efficace de population divisée par deux, augmentant les effets de dérive. Par ailleurs, la moindre efficacité de la recombinaison va augmenter la sensibilité aux interférences sélectives (sélection d'arrière-plan, balayage sélectif) et diminuer la probabilité de fixer plusieurs mutations avantageuses dans le même génome. En d'autres termes, l'autofécondation conduit à un fardeau génétique plus lourd, et diminue les capacités d'adaptation et l'efficacité de la sélection naturelle. On prédit donc que les espèces autofécondantes ont une probabilité d'extinction plus grande que les espèces allofécondantes – elles constituent un cul-de-sac évolutif.
Ces prédictions ont pour l'essentiel été évaluées chez des plantes, voire ne l'ont pas été du tout. L'objectif de cette thèse est d'apporter des éléments permettant de les tester chez des animaux, les escargots hermaphrodites d'eau douce. Pour ce faire, nous avons opté pour une approche d'évolution expérimentale permettant de contrôler régime de reproduction, conditions environnementales et pressions de sélection. Notre modèle d'étude est Physa acuta, une espèce allofécondante qui est capable de se reproduire par autofécondation et nous avons des lignées expérimentales se reproduisant soit en allofécondation stricte soit alternant avec une génération d'autofécondation depuis 20 à 30 générations au laboratoire. La première expérience montre que non seulement la dépression de consanguinité est largement purgée en une dizaine de génération d'autofécondation, mais aussi que le temps d'attente (un trait positivement corrélé au taux d'allofécondation) a fortement diminué. Nous n'observons en revanche aucune réallocation sur la fonction femelle. La deuxième expérience dans laquelle nous avons comparé la réponse à la sélection sur un trait morphologique en autofécondation et en allofécondation montre qu'une population en autofécondation répond d'abord mieux car les allèles sont progressivement placés à l'état homozygote mais cet avantage s'épuise rapidement probablement à cause des interférences sélectives car en trois générations elles commencent à répondre plus lentement que la même population en allofécondation (le trait considéré était la forme de la coquille). Ces travaux apportent des éléments nouveaux quant à notre compréhension de l'évolution de l'autofécondation, et proposent des éléments expérimentaux novateurs quant à la moindre adaptabilité des espèces autofécondantes.
Abstract : Many hermaphroditic organisms, either plants or animals, are able to reproduce by self-fertilization, at least alternatively with cross fertilization. Theoretical models predict several important consequences linked to this mating system. The first prediction is that a selfing population is less sensitive to inbreeding depression than an outcrossing one, because part of the depression can be « purged » meaning that the recessive deleterious alleles are easier to eliminate by natural selection under selfing. This purge creates a positive feedback to favour self-fertilization. In these circumstances, we also expect the evolution of traits facilitating self-fertilization (for example closed flowers) and a reallocation of resources from the male to the female function, because sexual selection is reduced in the male function. Self-fertilization also affects standing variation, as the effective population size is divided by two, enhancing the effects of drift. In addition, recombination becomes inefficient, increasing the extent of selective interference among loci (background selection, selective sweep) and decreasing the probability to fix several advantageous mutations in the same genome. In other words, self-fertilization decreases the adaptive potential and the efficiency of natural selection. We then predict that autogamous species have a higher probability of extinction, this is called the "dead end hypothesis".
Some of these predictions have been tested mainly in plants or not at all. The aim of this thesis is to test them in animals, using freshwater snails as model systems. To this end, we followed an experimental evolution approach using laboratory populations of Physa acuta a preferentially outcrossing snail able to reproduce by self-fertilization. These populations were maintained for 20 to 30 generations either under pure outcrossing or under alternating generations of outcrossing and selfing. In a first experiment we show that inbreeding depression is largely purged after only ten generations of selfing, but also that the waiting time, (a trait positively correlated to the outcrossing rate) decreased largely. We did not observe however any reallocation in favour of the female function. In a second experiment we compared the response to artificial selection on a morphological trait under selfing and outcrossing. We observed that when an outbred population switches to self-fertilization the response to selection is initially enhanced as alleles are progressively made homozygous. However this advantage is quickly offset by selective interference and after no more than three generations selfing populations start to respond to selection more slowly than outcrossing ones
This work brings new elements for the understanding of the evolution of mating systems, and provides empirical support for the lower adaptability of selfing species.